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An International Journal of Genetics

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Single Numbers, 14/- or $3 net. Annual Subscription, 40/- or $8.50 net

Printed and Published in Great Britain by OLIVER AND BOYD LTD.
Edinburgh: Tweeddale Court. London: 98 Great Russell Street, W.C.

HEREDITY VOLUME I PART II OCTOBER 1947

THE SPREAD OF A GENE IN NATURAL CONDITIONS IN A COLONY OF THE MOTH *PANAXIA DOMINULA* L.

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Received 19.iii.47

1. Introduction.
2. Description and Habits of the Moth.
3. The Dry Sandford Colony.
4. The Spread of the *medionigra* Gene.
5. The Population Numbers.
6. The Significance of Changes in Gene-Ratio.
7. Summary.
8. References.

(1) INTRODUCTION

THE spread of a gene in natural conditions is an event which repays detailed study since it provides an opportunity for examining evolution in progress. It has long been apparent to us that a careful watch should be kept for this occurrence and that whenever found it should, if possible, be analysed from two distinct points of view, ecological and genetic—a technique which has so far received much less attention than it deserves.

An instance of this kind has occurred in a wild population of the moth *Panaxia* (*Callimorpha*) *dominula* L. in the Oxford district, and we have been examining it from these two aspects for the last eight years. The experimental breeding is still in progress, and several more seasons must elapse before it is possible to report upon it in detail (it is briefly referred to on p. 167 of this paper), but the time has now come when an account can be given of the ecological side of the work, though we hope still to continue it.

We are grateful to Mr R. F. Bretherton who has on several occasions helped with the work of collecting, and to Mr W. H. Dowdeswell who took part with us in catching the 1939 sample; also to Mr T. C. Carter and Mr C. I. Rutherford who undertook a large part of the field-work in 1946. We are much indebted to them for their care and the very considerable time which they devoted to the collecting. Though, as will be explained on p. 155, the results for 1946 are reserved for discussion on a future occasion, the frequency

of the *medionigra* gene in that year is included in table 1 of this paper. We wish to express our thanks to Mr S. Beaufoy for his skill in taking the colour-photograph from which plate I has been made.

(2) DESCRIPTION AND HABITS OF THE MOTH

Panaxia dominula L. belongs to the family Arctiidæ. For the purpose of this paper it is necessary only to give a brief description of the insect itself and of its life-history and habits. Detailed information on these subjects may be obtained from Kettlewell (1942).

The chief food of the larva is Comfrey, *Symphytum officinale* L.; but it also eats a number of other plants, especially Dead Nettle, *Lamium album* L., and Stinging Nettles, *Urtica dioica* L. and *U. urens* L. on which it may be reared. The imagines appear in July, and a single female usually lays between 200 and 300 eggs. They are smooth, round with a flattened base, and being unprovided with any means of attachment they are scattered, and lie, loose among the herbage.

The young larvæ begin to feed in late July or in August and hibernate when partly grown, usually in the third instar, hiding themselves in dead curled-up leaves. They begin feeding again in March and complete their growth in late May or early June. They are hairy, blackish in colour, with yellow markings on the back and sides. Kettlewell (*loc. cit.*) points out that they are dimorphic, some having a pattern of small white dots which is absent from others. The proportions of the two forms appear to differ widely in different localities, and intermediates between them are rare or absent.

Pupation takes place in a loose silken cocoon spun among rubbish on the surface of the ground, or just below the soil. In captivity emergence may be much in advance of the normal time, the perfect insects appearing even in May.

The larvæ seem to be free from parasitism by Ichneumonoidea, but the pupæ are frequently killed by the Chalcid *Pteromalus puparum*. Perhaps a more serious danger is a virus disease which may destroy large numbers of larvæ in their last instar and sometimes does much damage to laboratory stocks. However, mice are probably the greatest menace of *P. dominula* in the colony which we studied. They are very abundant in the locality and almost certainly eat a considerable proportion of the pupæ, though the larvæ must largely escape their attacks. Thus it will be seen that this species is chiefly eliminated in the late larval and the pupal stages.

The imago is rather variable in size, but on the average measures 48 mm. across the expanded wings; see plate I, fig. 1. The ground-colour of the head, thorax and fore wings is deep bronze-green. The two pale marks on the thorax are a light yellow, and the spots on the fore wings are white, except for three which are normally yellow. These are the upper of the two basal spots, and that

immediately beyond it (that is to say, the central spot; the one which is reduced or absent in the heterozygous variety *medionigra*, p. 148), also the narrow spot towards the base of the inner margin. The abdomen and hind wings are scarlet, marked with black as indicated in the figure.

The moth flies actively in the sunshine with a characteristic undulating flight which makes it rather difficult to catch. Often it rises to a considerable height and may be seen circling the tree-tops. It also sits on the herbage and is especially attracted by the flowers of Hemp Agrimony, *Eupatorium cannabinum* L. In warm weather it is, in general, easily disturbed when resting. Towards evening it usually becomes sedentary, though large numbers may occasionally be seen flying round the tops of certain favourite trees at that time, if the temperature be high. The insect flies little on dull wet days. It seeks shelter from rain by hiding under leaves and low-growing herbage, but even then it is not difficult to find. We have ourselves made large catches in drenching rain by crawling through the vegetation and boxing the specimens as they sit.

Hatching from the pupa usually takes place in the morning, and numbers of males assemble to each virgin female, attracted by her scent, and dance up and down in the air in her immediate neighbourhood before one of them finds her. It is probable that the females almost always copulate on the day of their emergence, indeed we have repeatedly seen them doing so before their wings were dry; they begin to lay their eggs soon afterwards. Kettlewell (*loc. cit.*) concludes, we think correctly, that the females do not fly until they have laid a considerable proportion of their eggs, which they probably do within the first twenty-four hours after fertilisation. On the whole, it seems unlikely that the females fly until the day after they have emerged, and consequently that they always scatter a large number of their eggs round the plants on which they themselves had fed. However, it will be shown (pp. 159-62) that the average life of the individual can be estimated as 6·25 days, so that the proportion of the two sexes does not greatly differ from equality among the specimens which are active, whether readily disturbed or caught upon the wing.

When the moths fly they show their scarlet hind wings and display a typical "warning coloration"; but the pattern of the fore wings makes them rather inconspicuous at rest, particularly on a flower head. We have occasionally seen the imagines pursued and caught by birds, rather more often by dragon-flies, but neither event seems common. We ourselves do not find them unpleasant to eat, though it could always be maintained that they are distasteful to predators; certainly they may be less palatable than other food as easily, or more easily, secured. Some specimens are unwilling to fly after capture, or when they have spent some time in the dark. If touched, they will then display the scarlet hind wings and produce two large drops of clear fluid from the front of the prothorax, just behind the

head. It would be interesting to investigate the glands which give rise to this substance. To us its flavour is bland, certainly not repellent.

The chief habitats of the moth are river banks and inland marshes, though it is found in one quite exceptional locality on the coast of Kent. In such situations it usually forms localised colonies, often widely separated from one another. Within them the insects may be very abundant, though they seldom stray beyond their confines. In Britain the species is limited to England, where it is found from Kent to South Devon. Its main area of distribution lies south of the Thames, though it has been taken as far north as Staffordshire.

(3) THE DRY SANDFORD COLONY

The colony which forms the subject of this investigation is situated near the villages of Dry Sandford and Cothill in Berkshire, about five miles from the city of Oxford. It occupies a marsh the nature

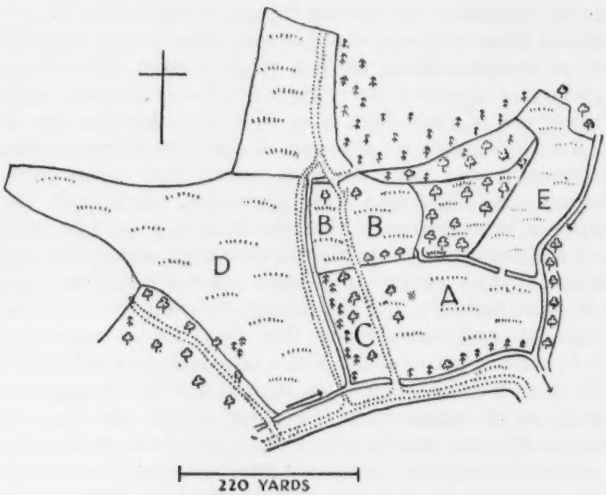


FIG. 1.—Map of the marsh at Dry Sandford, Berkshire.

The colony of *Panaxia dominula* occupies the areas A. to E. The species is most abundant in the western section of A and throughout B, also among the trees and in the open spaces in C up to the Conifers. Specimens are also to be found sparingly along the eastern border of D, only occasionally straying to the middle of it. They are rather scarce in area E.

The villages of Dry Sandford and Cothill lie respectively close to the east and the south sides of the marsh. Except where other indications are given, the site is bounded by agricultural land.

* = releasing point (in area A).

of which is exceptional in that district, in some ways resembling the fens (fig. 1). This has formed in a shallow basin partly fringed with woods. Some of the area is occupied with birch scrub, and a

narrow belt of pines traverses it on ground which is drier than the rest. The remainder of the site is that colonised by *Panaxia dominula*, and this comprises in all about 20 acres. One portion has perhaps always been fen, and bears traces of ancient peat cuttings (Clapham and Clapham, 1939). The rest has reverted to marsh land after attempts at former cultivation, so that the remains of hedges subdivide it. This non-wooded region falls into two types from the point of view of vegetation, and both are favoured by *P. dominula*. One part is relatively open and bears coarse grass in large hummocks, which are a great barrier to rapid progress. It is invaded to a varying extent by bushes of alder and birch. The other consists of a dense jungle of reed, *Phragmites communis*, shoulder high and more in July, together with plants of *Symphytum* and *Eupatorium* which are found in the more open ground as well. The *Symphytum* is common also along the banks of several ditches which intersect the site and drain into two small streams which border it.

The Dry Sandford area is surrounded on all sides by woods or agricultural land totally unsuited to *Panaxia dominula*, which is strictly confined to the marsh. Only two other localities for the moth have been reported in the Oxford district. One of these, where it is now apparently extinct, was situated at Tubney about 1½ miles away. Very few specimens from this colony exist in collections, and it is not known whether it had the special characteristics of that at Dry Sandford. Owing to the nature of the intervening country, the passage of stray specimens from one colony to the other must have been a rare event, but indeed it would not have affected the results of the present work. The remaining colony to which reference has been made is near Weston-on-the-Green, Oxfordshire. This is about 13 miles from Dry Sandford, with the city of Oxford lying between.

The evidence is rather strong that these are the only colonies of the moth in the district. The entomology of the country surrounding Oxford is as well known as any in the Kingdom. It has been worked by generations of undergraduates collecting Lepidoptera, and there have been several resident entomologists in the city throughout the present century, and long before. *Panaxia dominula*, a large day-flying Arctiid with scarlet hind wings, is among the most conspicuous of all British moths. Moreover, it is sufficiently local to be an object of much interest to the collector, and it is hardly credible that other colonies of it should exist close to Oxford without being known. Similarly, were specimens from Dry Sandford frequently to leave the marsh and stray over the surrounding country, contrary to their well-known habits, the fact would almost certainly have been discovered, by others if not by ourselves.

It is clear, therefore, that this colony is an isolated one; a conclusion which receives some support from the fact that var. *medionigra* (p. 148), which in 1945 occupied 11·8 per cent. of the population there, is unknown elsewhere. This, however, is but poor

evidence of isolation, since mutation to the gene concerned is doubtless occurring throughout the whole species, at the low rate consistent with particulate inheritance, and its absence in other colonies is probably due to its elimination there, conditions being unfavourable for it except at Dry Sandford.

(4) THE SPREAD OF THE *MEDIONIGRA* GENE

Two varieties of *Panaxia dominula* are recorded only from the Dry Sandford colony, and they must be of great rarity elsewhere. These are *medionigra* Cockayne (1928), the frequency of which has varied between 7·9 and 20·5 per cent. of the population during the period 1939 to 1946 inclusive, and *bimacula* Cockayne (*ibid.*), which is much rarer. They have now been bred extensively by several entomologists including ourselves, and it has been shown that they represent the heterozygous and homozygous expression of the same gene.

The homozygote, *bimacula*, is illustrated on plate I, figs. 7 and 8. It will be seen that it differs from the typical form (plate I, fig. 1) in a great extension of the black pigment. This replaces the scarlet on the hind wings to a considerable degree and obscures all the spots on the fore wings except the two basal ones and that on the inner margin. These three marks must be under a different genetic control for they are not reduced at all, though they may disappear in other varieties. The small white dots within the outer margin are present in some specimens of *bimacula*, absent from others. It should be noticed that the pale spots on the fore wings are replaced by black, not by an extension of the dark metallic green ground-colour ; their position can therefore be determined by careful inspection.

The heterozygotes seem always distinguishable as var. *medionigra* (plate I, figs. 3-6) in wild material, but they resemble the typical form more closely than *bimacula*. As is general, and indeed to be anticipated, in genetic studies, the rare homozygote is more variable than the common one and the heterozygote is more variable still. Typically, *medionigra* differs from the normal form in the absence of the central (yellow) spot on the fore wings, and in the presence of an additional black spot near the centre of the hind wings. Both these characters are subject to much variation. The central spot on the fore wings may be present though reduced in size, but occasionally it is as large as in the ordinary homozygous *dominula*. Sometimes also the extra spot on the hind wings may be reduced to a few black scales surrounded by a small area of yellow ones which serve to indicate its existence more clearly. Now and then, on the other hand, it is so large as to become confluent with the black spot above and below it. Plate I, figs. 3 and 4 represent the less extreme, and plate I, figs. 5 and 6 the more extreme forms of var. *medionigra*.

Collecting in Dry Sandford during 1936 and 1938, when *P. dominula* was on the wing, we noticed several specimens of *medionigra*, and it

occurred to us that the variety was more frequent than it had been in 1921, when we found no examples of it though the moth was common. The circumstance appeared to be of sufficient interest to warrant further examination, and received some support from a cursory survey of some old local collections.

These indications lead us to study the situation more thoroughly. In the first place, we endeavoured to determine the frequency of *medionigra* in former years, but it was difficult to obtain a sufficient number of specimens for the purpose. We, of course, took account only of those which were dated,* and many of these were merely labelled " Oxford " or " Oxford District." Owing to the existence of the two other localities in the neighbourhood, to which reference has been made, such insects were useless for our purpose ; we had to reject all those whose data did not specifically state that they were caught at Dry Sandford or Cothill, being the two villages bordering our locality. We examined a considerable number of collections in private hands, and in museums, in which specimens of *P. dominula* from Dry Sandford were likely to be preserved, and some contained short sets of them. It chanced that none of the insects to which we had access had been caught during the period 1929-1933, and a few only were of more recent date. As a preliminary step, we therefore decided to determine the frequency of the *medionigra* gene in specimens captured up to 1928, then ten years before, and to obtain an estimate of it the following season, that of 1939, for comparison.

After much search, we were able to accumulate 168 specimens which had been caught before 1929, and only 4 (2·4 per cent.) of them were *medionigra*. The value of this evidence requires careful consideration. Variety hunting has long been one of the chief aims of those who collect Lepidoptera, in many cases to an extent disproportionate to its real interest. It is customary, therefore, for collectors to catch a short series only of typical specimens from a given locality, but to amass all the varieties that they can obtain, unless they be very common ones. Most collections of Lepidoptera, therefore, do not represent a random assortment of each species but one which much exaggerates its variability. There is certainly no tendency for collectors to reject abnormal individuals ; a fact which anyone acquainted with butterfly and moth collecting in this country will readily endorse. Thus the sample of 168 specimens captured prior to 1929, contained in ordinary collections, will tend to overestimate, certainly not to under-estimate, the frequency of var. *medionigra*.

In 1939 we secured 223 specimens. These were a random sample in the sense that we took every individual we could catch, irrespective of form or condition. The species was unusually common that year,

* Together with some undated specimens from the colony which were known to have reached museums in bequests received prior to 1929.

and this total was obtained by three of us working throughout a single day. The moths were kept in boxes until evening, when all were released except for a few which were required for collections. There was no possibility, therefore, of counting a specimen twice. The catch comprised 184 typical *dominula*, 37 *medionigra* and 2 *bimacula*. The heterozygotes thus occupied 16·6 per cent. of the population, and the gene-frequency was 9·2 per cent., being 7·7 times greater than in the earlier sample in which, if anything, it would be exaggerated. There can be no doubt that this represents a genuine increase, and that the gene had spread in nature. Consequently, it seemed worth while to obtain an estimate of its frequency in each succeeding year.

The moth has not again been so common as it was in 1939, and from then, until 1946, only one or occasionally two people have been available for the work of collecting. Consequently, it has been impossible to catch our sample on a single day ; nor would this have been practicable when we began estimating the population numbers from 1941 onwards. In order to ensure that the same individual should not be counted again if caught on a subsequent occasion, each specimen taken was marked with cellulose paint (see p. 151 for the details of this technique). The results of this work are given in table 1, and they

TABLE 1

The distribution of the medionigra *gene among specimens caught up to 1928 and among those caught from 1939 to 1946*

Year	*dominula*	*medionigra* (heterozygotes)	*bimacula*	Total	Gene-frequency (%)
Up to 1928	164	4 (2·4%)	...	168	1·2
1939	184	37 (16·6%)	2	223	9·2
1940	92	24 (20·5%)	1	117	11·1
1941	400	59 (12·8%)	2	461	6·8
1942	183	22 (10·7%)	...	205	5·4
1943	239	30 (11·2%)	...	269	5·6
1944	452	43 (8·7%)	1	496	4·5
1945	326	44 (11·8%)	2	372	6·5
1946	905	78 (7·9%)	3	986	4·3
1939 to 1946	2781	337 (10·8%)	11	3129	5·7

will be discussed in section 6 of this paper. It should, however, be noticed at this stage that the frequency of the *medionigra* gene had greatly increased between the old samples, taken up to 1928, and that obtained in 1939, while the increase, which of course may have been very variable, was still evident the following year. In 1941, however, the gene-frequency dropped from 11·1 to 6·8 per cent. of available loci, and has subsequently shown a tendency to fluctuate round a mean of 5·2 per cent., rather than to return to the high level attained in 1939 and 1940 (9·2 and 11·1 per cent.), or to sink back to the low figure of 1·2 per cent. observed prior to 1929.

In the total sample of 3129 caught specimens, the number of *bimacula* corresponds closely with expectation based upon the number of heterozygotes (10·24 expected, 11 observed), so that our data do not of themselves suggest a differential viability between the two varieties. On the other hand, this is inherently possible, while the error involved is great when so few *bimacula* are expected and a considerable differential viability could exist without becoming apparent in our captures. Indeed we believe that *bimacula* is distinctly less active than the other two forms, an impression which Dr H. B. D. Kettlewell, who has an extensive experience of this insect, is prepared to endorse. It is quite probable, therefore, that in this colony the heterozygotes are at an advantage compared with either homozygote, a situation which must necessarily lead to polymorphism.

As will be explained, we have decided not to undertake the full discussion of the last sample, that of 1946, in this paper. However, we have included the gene-frequency of *medionigra* for that year to indicate that, though it is by a small margin the lowest since we began to study the colony in 1939, there is no evidence that in 1946 it differs materially from its values in the previous five years.

(5) THE POPULATION NUMBERS

After taking the 1940 samples, it had become clear that a situation of sufficient interest had developed to warrant estimating year by year the absolute numbers of the population in which the *medionigra* gene was spreading. Accordingly we began this work in 1941 and have continued it each season since, using the method of marking, release and recapture, developed by Dowdeswell, Fisher and Ford (1940). That paper should be consulted for fuller details of the technique, which are only given here in brief.

The specimens are each placed in separate boxes when caught, and at the end of the day's collecting they are marked with cellulose paint. This has the property of very rapid drying and seals the scales on to the wing membrane. It is then permanent and waterproof. A dot applied with a sharpened stick is sufficiently hard in about ten seconds for the insect to be returned to its box. Only the upperside of the fore wings is marked, for this can be seen without disturbing the insect when at rest. Moreover, the hind wings are the more easily damaged, and a spot of paint placed upon them might be smeared if not quite dry, since they are folded and are overlapped by the front pair. The colour of the paint and its position on the wings, left or right, basal or distal, indicates the date. We used four colours: red (this colour being absent from the fore wings), bright green, pale blue and, when necessary, purple, and we found them easily distinguishable without risk of confusion. A number of marks can be applied to a single insect, so that it carries a dated record of its first capture and of any subsequent recaptures. One of the

marked specimens used in our work is shown on plate I, fig. 2. The three spots of paint on the fore wings, differing in colour and position, each indicate a date of capture and release.

The majority of the specimens sit quietly while they are being marked, for even those which were active when caught tend to become quiet after spending some time in the dark. A certain number, however, are very lively, and their wings have to be held lightly but firmly with a pair of forceps when the paint is applied and while it dries. This can generally be done without injury, but occasional specimens were damaged in the process. These were always killed ; a fact which could be taken fully into account in the records and calculations. It would of course have introduced an error to return damaged specimens to the population. For the same reason, injured or deformed specimens which were sometimes found sitting on the herbage, were not released.

The validity of our technique depends upon the free distribution of marked specimens throughout the colony, in the sense that the chances of recapturing specimens bearing a certain date-mark must be proportional to their frequency in the population at the time of recapture. This we endeavoured to secure in the following manner.

The marked insects were all taken to a central releasing point and there liberated. But, as already mentioned, we returned to the population only such specimens as appeared capable of distributing themselves freely among it, and we allowed them an adequate opportunity for doing so. If a sample were taken on successive days, work was not restarted until the insects had been flying freely for several hours since the previous release.

Marked specimens were in fact caught at the furthest limits of the colony in all directions from the releasing point even on the next day. The rather wild erratic flight of this species seems to make it but little influenced by the direction of the wind. Yet there can be no doubt that we obtained relatively more marked examples close to than far distant from the releasing point. Therefore we randomised our own captures as far as possible, spending approximately equal periods of time each day that samples were taken catching in various parts of the colony.

We also gave careful consideration to the possibility that some marked insects might have been injured without our noticing the fact, and were for this or any other reason (including what, for want of a better term, might be called a psychological one) incapable of distributing themselves throughout the colony. Accordingly, when released, the insects were thrown well up into the air, when the great majority, even of those previously sitting, would begin to fly as they fell and would be seen to scatter widely. A few, however, came down near at hand. There was good reason to think that some at least of such specimens would become active later (p. 165), so that it seemed best to make the following arbitrary decision in regard to capturing

methods. That is to say, no collecting was done within a radius of ten yards from the releasing point. This area was, however, carefully examined at the beginning of the work on each occasion, and marked specimens, usually only one or two, were sometimes, but not always, found within it. These were not counted among the recaptures, and if on throwing into the air again they still fell within the ten yards radius, they were killed and entered in the table as if they had been destroyed at the time of release.

The results of this work are presented in the triangular tables (tables 2 to 6). These are arranged like a trellis. The dates form a horizontal row along the top, and from each of them lines descend forwards and backwards at 45° so as to interesect. The total captured

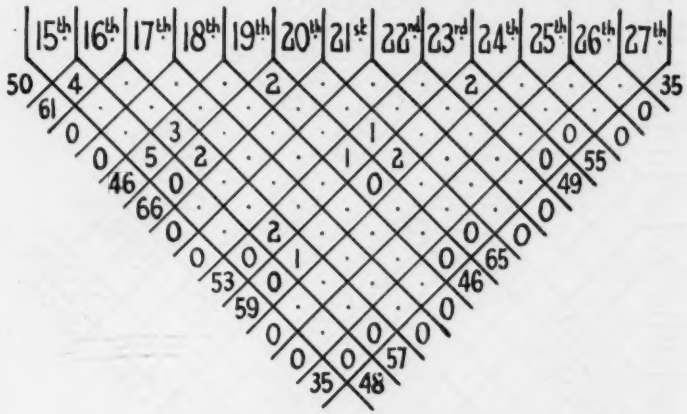


TABLE 2.—Captures, releases and recaptures of *P. dominula* in 1941.

on a given day is entered at the end of the line running south-west from the date in question, and the total released is entered at the end of that running south-east. The number released may equal that captured or it may be less for, as previously mentioned, a few specimens may be damaged in the marking, while some may be required for other purposes and not returned to the population. The recaptures are entered in the body of the table. The figure 0 indicates that no specimens marked on a given previous date had been recaptured on a certain subsequent date. A dot (.) shows that a recapture was impossible on the occasion indicated, either because no insects were being caught that day or because none had been released on the previous date in question.

Consider, for example, table 4 for 1943. About the middle will be seen the entry 5. This means that of 46 specimens released on the 14th July, 5 were recaptured on the 18th among a sample of 50, of which 48 were then released. It will also be seen that though 47

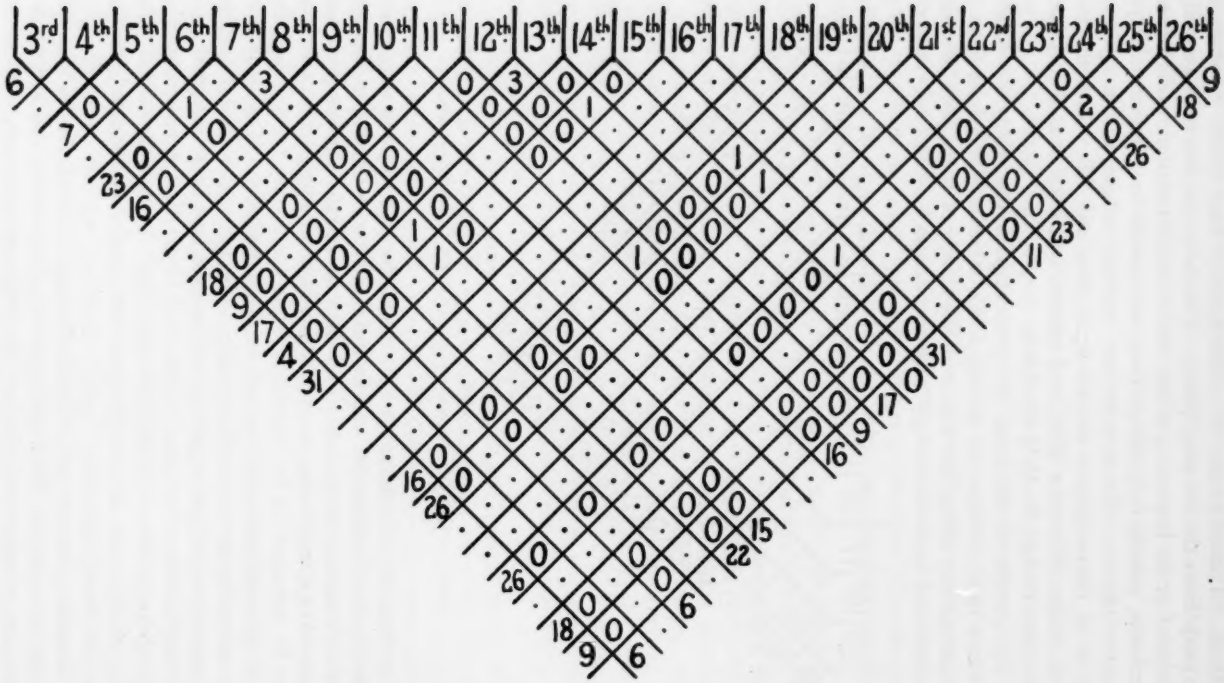


TABLE 3.—Captures, releases and recaptures of *P. dominula* in 1942.

were released on the 15th, none of them was retaken among the sample of 29 obtained on the 19th. The row of dots passing downwards in both directions from the 17th shows that no collecting was done on that date.

The detailed analysis of these tables may now be described, but we have decided in the present paper to carry this only up to the end of 1945. The work upon the population numbers for 1946 will require further study which would hold back the present report unduly, while it is already evident that it will not substantially alter our present findings. We hope to acquire additional data for a few more years and to publish such further results, together with the findings for 1946, at a later period.

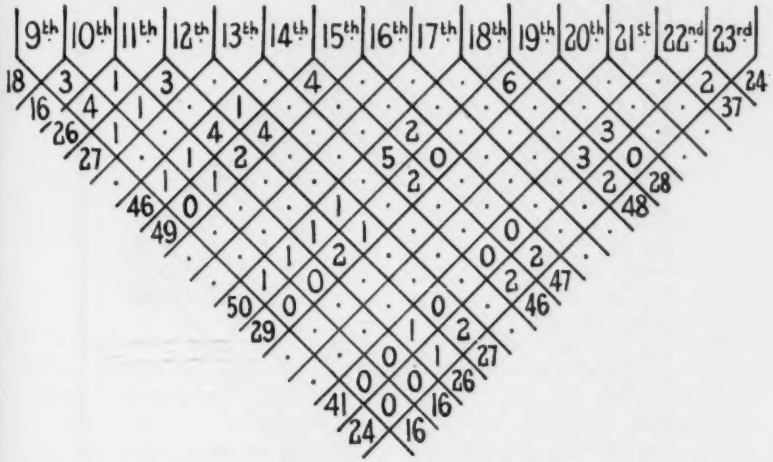
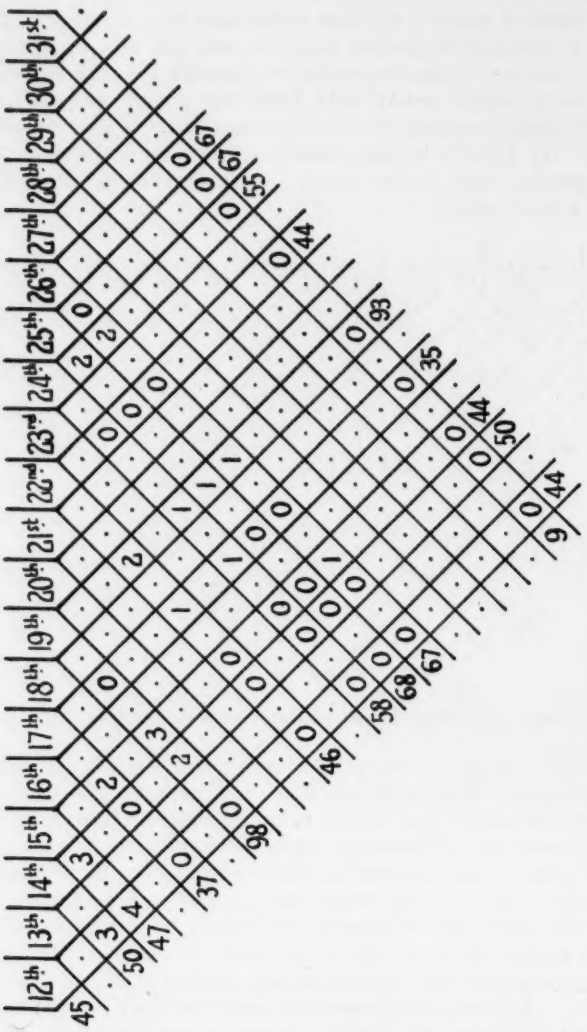


TABLE 4.—Captures, releases and recaptures of *P. dominula* in 1943.

The trellis diagrams described above give a complete account of the observational data in respect of releases, captures and recaptures, with the reservation that when, as occasionally happens, the same individual is recaptured more than once its identity, though recognised in the original record, cannot be reconstructed from these diagrams. This is a point of small importance, since not only are such individuals few, but the additional information could only be utilised in conjunction with knowledge of the manner in which the death-rate in the wild population changes with increase of age, and such knowledge we do not possess. Although it is probable *a priori* that there is some increase in the rate of elimination with increasing age from the time of emergence, the adoption of any other table of mortality than that provided by a constant death-rate is almost completely precluded by the fact that the insects are of unknown age at the time of their first capture and release. Moreover, as will be shown, expectations

based upon a constant death-rate are, in fact, somewhat closely verified in the data for the first five years (table 13), showing that in the portion of the life-span most influential upon the actual fre-


12th
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29th
30th
31st
45
3 50
3 4 47
2 0 37
3 2 0 98
2 3 0 46
0 0 0 58
0 0 0 68
0 0 1 0 67
2 1 0 0 .
1 1 0 0 .
0 0 0 0 .
2 2 0 0 .
0 0 0 0 .
0 0 0 0 .
0 0

from later observed recapture. It has been found, as the following tables (especially table 13) demonstrate, that the observed difference in the distribution of time interval for these five years agrees somewhat closely with expectation on the basis of a daily elimination of 16 per cent. from the population available for capture. This corresponds with an expectation of life of 6¼ days, a value entirely consonant with experience of the moths in captivity.

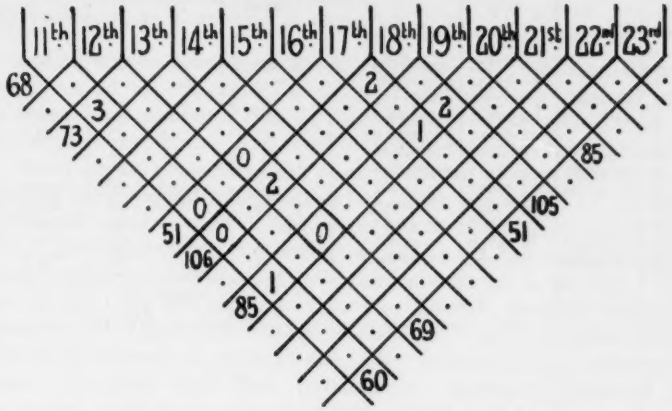

TABLE 6.—Captures, releases and recaptures of *P. dominula* in 1945.

Since the calculations are of a type which have not, we believe, been previously used or exhibited, it will be necessary to explain them at some length, and to show the arithmetical procedure in detail (tables 7 to 11). In the record for 1941, for example, captures and releases were made on seven occasions, of which the earliest was the 15th and the latest the 27th of July. There is thus the possibility of recapture at intervals from one to twelve days. The dates of release are shown in the left-hand column, followed by a column showing the days' interval from the date of release to 27th July. If this is r days, the proportion of moths released which at the survival rate adopted would survive until 27th July is $(\cdot 84)^r$. This fraction multiplied by the number actually marked and released on the six previous occasions supplies the numbers in the third column, and at any intermediate date, *e.g.* s days, before 27th July the numbers expected to be available for capture will be the sum of these entries for releases prior to that date, divided by $(\cdot 84)^s$.

Consequently, in the fourth column the entries are progressive sums from the bottom of the table, and these values divided by $(\cdot 84)^s$ give the estimated numbers of marked insects flying at any date. In this reckoning it may be noted that any insect already marked twice will be counted twice, so that if it is in fact recaptured it should be counted as so many recaptures as it bears marks.

Daily survival 84 *per cent.*

TABLE 7

1941

July	r	Released. r^1			Total recaptures	Total days survived	Excess over expectation	Total captures	Estimated number	July
26	1	...	101·87554	584·06462	0	0	0	35	∞	27
25	2	...	101·87554	482·18908	...	...	...	...	...	...
24	3	32·59872	101·87554	380·31354	...	...	...	...	...	...
23	4	24·39570	69·27682	278·43800	5	18	−2·010	59	1379·2	24
22	5	...	44·88112	209·16118	4	21	2·359	53	1194·4	23
21	6	...	44·88112	164·28006	...	...	...	...	...	...
20	7	19·18087	44·88112	119·39894	...	...	...	...	...	...
19	8	11·40229	25·70025	74·51782	4	10	−1·598	66	1437·0	20
18	9	...	14·29796	48·81757	8	29	1·686	46	331·7	19
17	10	...	14·29796	34·51961	...	...	...	...	...	...
16	11	8·37427	14·29796	20·22165	...	...	...	...	...	...
15	12	5·92369	5·92369	5·92369	4	4	0	61	614·9	16
							0·437			

TABLE 8

1942

July	r	Released. r^1			Total recaptures	Total days survived	Excess over expectation	Total captures	Estimated number	July
25	1	15·12000	51·84136	266·75526	0	0	0	9	∞	26
24	2		36·72136	214·91390	2	4	−7·705	18	393·4	25
23	3	15·41030	36·72136	178·19254	...	...	...	...	...	...
22	4	...	21·31106	141·47118	1	8	1·362	26	934·8	23
21	5	...	21·31106	120·16012	...	...	...	...	...	...
20	6	8·07985	21·31106	98·84906	...	...	...	...	...	...
19	7	3·24599	13·23121	77·53800	2	6	−5·720	26	489·6	20
18	8	...	9·98522	64·30679	2	12	−0·880	16	270·7	19
17	9	...	9·98522	54·32157	...	...	...	...	...	...
16	10	...	9·98522	44·33635	...	...	...	...	...	...
15	11	4·55443	9·98552	34·35113	...	...	...	...	...	...
14	12	...	5·43079	24·36591	2	10	1·027	31	573·0	15
13	13	1·76230	5·43079	18·93512	1	7	3·487	4	176·0	14
12	14	·78370	3·66849	13·50433	3	3	−8·044	17	200·5	13
11	15	1·17033	2·88479	9·83584	0	0	...	9	∞	12
10	16	...	1·71446	6·95105	0	0	...	18	∞	11
9	17	...	1·71446	5·23659	...	...	...	...	...	...
8	18	·65031	1·71446	3·52213	...	...	...	...	...	...
7	19	·80118	1·06415	1·80767	3	3	−2·096	16	130·9	8
6	20	...	·26297	·74352	1	2	−0·827	23	166·1	7
5	21	·15418	·26297	·48055	...	...	...	...	...	...
4	22	...	·10879	·21758	0	0	...	7	∞	5
3	23	·10879	·10879	·10879	...	...	−19·396			

We shall, however, also be interested in the time intervals between marking and recapture. Column five shows the values of column four summed a second time from the bottom, so that after an interval of t days every marked insect is counted t times, thus allowing of the estimate of the total number of days between marking and recapture to be expected among those recaptured at any given date. For example, on 24th July the average number of days between marking and recapture at that date is expected to be $\frac{278{\cdot}43800}{69{\cdot}27682}$, and this multiplied by the number of actual recaptures on that date, namely five, gives an expectation of 20·010, which may be compared with the actual total number of days for these insects, namely 18. The excess over expectation, in this case −2·010, is shown in the eighth column.

To make an estimate of the numbers flying at any date we may multiply the expected numbers marked in the population by the total number of captures, and divide by the number of recaptures at that date. We thus obtain the estimates shown in the tenth column, corresponding to each date of collection subsequent to the first. The rows of the table are redated in the last column, since the numbers captured at any date have references only to releases at previous dates, while those released on the same day have references only to subsequent dates. The fact that these numbers are estimates, obtained by calculation, and not counts, has been emphasised by retaining one place of decimals.

The estimated rate of elimination, 16 per cent. daily, has been arrived at by making the observed discrepancy between the total days survived, and that expected, vanish approximately over the whole period of five years. The comparison is made in table 12. For three years the totals agree closely, but it may be noticed that survival is below expectation in 1942, and in excess in 1943. The former was a year of prolonged emergence and unfavourable weather, while in the latter, though total numbers are still low, the weather was favourable, and the period of emergence somewhat short. That 1943 was a favourable year is also indicated by the great increase in numbers observed in 1944 (table 15).

On occasions in which no marked moth was captured, the method of calculation described gives an infinite estimate of the numbers available. This happens frequently on the last day of the record, when the numbers available for capture have usually been much diminished, and sporadically whenever it has been impossible to catch more than a few moths. In view of the stability of the other entries we do not think these cases will cause any misapprehension. It is, however, a puzzling feature of our experience that the estimates at the later dates of collection do so often indicate the highest of the estimated numbers. For example, in 1944, numbers in the neighbourhood of 3000 are estimated for three successive days, 24th-26th July, while by 31st there were very few moths available. Apart from any

systematic errors in the method of estimation the data would seem to imply a large increase in the death-rate towards the end of the

TABLE 9

1943

July	r	Released. r^4			Total recaptures	Total days survived	Excess over expectation	Total captures	Estimated number	July
22	1	31·08	96·54997	489·82118	11	80	24·194	24	210·7	23
21	2	...	65·46997	393·27121	7	32	−10·048	41	456·5	22
20	3	...	65·46997	327·80124	...	...	...	...	...	...
19	4	13·94040	65·46997	262·33127	...	...	...	...	...	...
18	5	20·07417	51·52957	196·86130	11	39	−3·024	29	272·9	19
17	6	...	31·45540	145·33173	11	56	5·177	50	341·9	18
16	7	...	31·45540	113·87633	...	...	...	...	...	...
15	8	11·65017	31·45540	82·42093	...	...	...	...	...	...
14	9	9·57792	19·80523	50·96553	11	29	0·693	49	355·9	15
13	10	...	10·22731	31·16030	7	23	1·673	46	322·8	14
12	11	3·96676	10·22731	20·93299	...	...	...	...	...	...
11	12	3·20867	6·26055	10·70568	5	8	−0·550	27	230·1	12
10	13	1·65863	3·05188	4·44513	5	9	1·717	26	128·6	11
9	14	1·39325	1·39325	1·39325	3	3	0	16	71·7	10
							19·832			

TABLE 10

1944

July	r	Released. r^4			Total recaptures	Total days survived	Excess over expectation	Total captures	Estimated number	July
30	1	...	98·36094	775·35552	0	0	0	9	∞	31
29	2	...	98·36094	676·99458	...	...	...	...	...	...
28	3	...	98·36094	578·63364	...	...	...	...	...	...
27	4	...	98·36094	480·27270	...	...	...	...	...	...
26	5	28·02120	98·36094	381·91176	...	...	...	...	...	...
25	6	23·53697	70·33974	283·55082	4	22	5·875	67	2817·2	26
24	7	16·22997	46·80277	213·21108	3	8	−5·667	68	3019·8	25
23	8	...	30·57280	166·40831	2	12	1·114	58	3004·5	24
22	9	9·16149	30·57280	135·83551	...	...	...	...	...	...
21	10	...	21·41131	105·26271	3	11	−3·749	46	1576·8	22
20	11	...	21·41131	83·85140	...	...	...	...	...	...
19	12	11·47716	21·41131	62·44009	...	...	...	...	...	...
18	13	...	9·93415	41·02878	5	22	1·350	98	1577·7	19
17	14	3·04774	9·93415	31·09463	...	...	...	...	...	...
16	15	...	6·88641	21·16048	2	4	−2·146	37	1463·0	17
15	16	2·70347	6·88641	14·27407	...	...	...	...	...	...
14	17	2·58058	4·18294	7·38766	7	15	2·637	47	457·1	15
13	18	...	1·60236	3·20472	3	6	0	50	517·4	14
12	19	1·60236	1·60236	1·60236	...	...	0	...	...	...
							−0·586			

season, which is in itself not improbable. Trial calculations based not on a constant expectation of life, but on one decreasing so rapidly

as a half-day for each day of survival, do not appear to make any appreciable difference to the absolute or the relative numbers estimated as flying at different dates. It would appear, therefore, that the late occurrence of the largest numbers indicated by our tables is not probably due to any systematic error ascribable to the assumption of constant death-rate.

TABLE 11

1945

July	r	Released. r^4			Total recaptures	Total days survived	Excess over expectation	Total captures	Estimated number	July
19	1	...	137·17008	493·82486	4	16	1·600	85	2914·9	20
18	2	74·08800	137·17008	356·65478	...	...	...	...	...	...
17	3	30·22790	63·08208	219·48470	4	12	1·562	106	2369·2	18
16	4	...	32·85418	156·40262	0	0	0	51	∞	17
15	5	...	32·85418	123·54844	...	...	...	...	...	...
14	6	...	32·85418	90·69426	...	...	...	...	...	...
13	7	20·36123	32·85418	57·84008	...	...	...	...	...	...
12	8	...	12·49295	24·98590	3	6	0	73	1030·2	13
11	9	12·49295	12·49295	12·49295	...	...	...	...	...	...
							3·162			

When, as on 17th July 1945, no recaptures appear in a satisfactory catch, it is easy to make a joint estimate from the data of adjacent days, such as 17th and 18th of that year, from which it appears that the estimated number 2369·2 for 18th July would be raised by the experience of 17th to 3076·0. It is thus probable that on these days the numbers were as many as, if not more than, on 20th July.

TABLE 12

	Expected	Observed	Difference
1941	81·563	82	+0·437
1942	74·396	55	−19·396
1943	259·168	239	+19·832
1944	100·596	100	−0·596
1945	30·838	34	+3·162
	546·561	550	+ 3·439

That the death-rate adopted is in agreement with our general experience over these five years is shown by table 12, which shows for each year, and for the total, the numbers expected and observed for total days survival in the wild population of marked specimens recaptured. On the total data the number observed is more favourable, but by less than 1 per cent., than the value expected. It may be

mentioned that in 1946 survival was again below expectation, as in 1942, so that had all six years been used the average mortality would have been somewhat heavier than that which we have adopted.

A somewhat more searching comparison is also possible from tables 7-11; for, knowing the approximate numbers of surviving insects marked at each previous marking date we can calculate the numbers of each of these to be expected among a given total number recaptured. This enables us to reconstruct the actual numbers to be expected among recaptures bearing marks 1, 2, 3, ... days old. Table 13 exhibits this comparison for the total of five years' experience. Had there been a striking increase of death-rate with age in the life period of the insects actually affecting our record, it might be expected that although the mean interval between marking and recapture is brought to agreement as shown in table 12, yet that observations should fall short of expectations at the beginning and end of table 13, while being in excess for the middle entries. As table 13 shows, however, there is no sign of any disturbance of this kind, the observations at all intervals from one day to the longest survivals to be expected, *i.e.* ten days or more, showing, in fact, a striking agreement with expectations based on the known numbers released at previous dates.

TABLE 13

Distribution of intervals between marking and recapture compared with expectation on the assumption of a constant death-rate of 16 per cent. daily

5 years, 1941-45		
Days	Expected	Observed
1	41·292	41
2	21·499	22
3	25·295	25
4	23·295	25
5	14·152	13
6	4·202	2
7	8·194	7
8	6·292	9
9	3·279	4
10	1·765	0
11	1·301	4
12 and over	2·422	1
	152·988	153

Since the date of capture of all insects is known, the records provide material for testing whether the variant gene to which the forms *medionigra* and *bimacula* are due is associated in nature with earlier or later emergence than its normal allelomorph. Had only the two forms *dominula* and *medionigra* occurred in the records for each year, we should have been content to examine this point by a simple

comparison of the mean dates of (first) captures of these two forms. In several years, however, the form *bimacula* also was captured, and, although few in numbers, these specimens are particularly relevant to the question of date of emergence. At each date of capture, in fact, we are concerned with the observed gene-ratios in the insects caught, rather than the ratio only of the two commoner genotypes. From this point of view the question is closely analogous with that of the regression of gene-ratio on date of capture, with the reservation that since we know the numbers upon which each gene-ratio is based we need not rely upon the observed day-to-day variation to supply an estimate of its precision. The test may, in fact, be made by isolating a single degree of freedom from the observed frequencies of the three possible genotypes at all dates.

If in the record of any year the proportion of variant genes observed is p, and the date of a particular observation t, then the number of *bimacula* observed on any occasion has a coefficient $2(1-p)t$, the number of *medionigra* has similarly the coefficient $(1-2p)t$, and the number of *dominula* has the coefficient $-2pt$. To obtain χ^2 for the one degree of freedom concerned, the square of this component is divided by $2p(1-p)\Sigma n(t-\bar{t})^2$, where n is the total number caught at any date.

The comparison between the mean dates for the (first) captures of insects bearing these two allelomorphic genes is shown in table 14;

TABLE 14

	Normal gene July	Variant gene July	Difference d		χ^2	
1941	20·228	20·857	+0·629	later	3·6357	
1942	15·582	13·364	−2·218	earlier	2·3124	
1943	15·713	16·133	+0·420	later	0·2768	
1944	20·171	19·600	−0·571	earlier	0·5781	
1945	16·079	16·063	−0·016	earlier	0·0011	
1946	20·127	20·155	+0·028	later	0·0035	
					6·8076	$n = 6$

the χ^2 measure of significance is shown in the last column. It appears that of six years' experience the variant gene has been later in three and earlier in three, and that in four years out of six χ^2 is less than its average value unity. In 1941 and 1942 there are slight indications of a difference in the date of emergence; but these are in opposite directions, and evidently our experience gives no indication of any general association of the genes concerned with date of emergence. Nor do the data show any significant heterogeneity from year to year in this possible effect, since the total of χ^2 for six degrees of freedom comes to 6·8076, very close to its expectation.

It is, perhaps, worth little to draw attention to associations that

are not statistically significant, but it so happened in these six years that the range for the mean date of first capture is only 4·646 days for the normal gene, against 7·493 days for the *medionigra* gene. It would not be surprising if the variants were less thoroughly stabilised against environmentally induced disturbances of the date of emergence than are the typical insects.

To pass from estimates of the numbers available for collection, on days on which collection took place, to estimates of the total emergence of a whole season is a matter of some difficulty. If indeed estimates could be made every day for the whole period of about three weeks during which any of the moths are to be caught, the knowledge we have obtained of the average length of life in the field would give the total population of the year, by adding together the numbers available on each day, and dividing by 6½. However, collecting cannot be carried out every day, and in any case no good estimates are possible until a considerable population of marked insects has been built up. In these circumstances only rough and tentative estimates are possible.

For years in which the season was quickly over, such as 1941 and 1945, there are only a few days for which the numbers are well determined. In these years, however, it is reasonable to suppose that a large proportion of those emerging in the year were alive at the time the daily numbers were at their height. Thus the total emergence must have been about 2000 to 2500 in 1941, and about 4000 in 1945. In 1942 the season was unusually prolonged, moths could be caught for at least 23 consecutive days, but all estimates were small. The maximum may not have exceeded 500 ; on the other hand, the death-rate in this year may have been exceptionally high, and although the total emergence may not have exceeded 1200, it may have been as high as 2000. In 1943, on the other hand, catches were good, but for only 14 days ; the estimates show that numbers did not rise much above 300 to 400, but were well maintained. The total emergence for this year may have been no more than 1000. Finally, in 1944, the numbers were certainly over 1000 for something like a fortnight and exceeded 3000 at the peak. The total emergence must have exceeded 5000. These imperfect estimates, the result of the comparison of numerous tentative approaches, are summarised in the following table.

TABLE 15

Approximate estimates of numbers observed

1941	2000 to 2500
1942	1200 to 2000
1943	1000
1944	5000 to 6000
1945	4000
1946	6000 to 8000

An estimate for 1946 is added. This year the moths were exceptionally abundant for fully three weeks. For most of this time, however,

less than 2000 must have been flying. On the other hand, the death-rate seems to have been somewhat high, so that the total emergence might have exceeded 8000.

Two sources of error other than those so far discussed might be thought to affect these estimates of the absolute numbers of *Panaxia dominula* in our colony.

First, it has been suggested that a significant proportion of the specimens rarely fly but remain in a semi-quiescent state sitting about on the herbage throughout their lives. This, if correct, would tend to make our estimate of the population too low, were our samples derived predominantly from insects caught on the wing. But that was not so : not only were they netted as they flew of their own accord or were disturbed, but a careful search was made for those resting on herbage or under leaves, and on wet days almost the whole of our samples had to be obtained in that way. In addition, we attacked this suggestion experimentally in the following manner during the years 1944 and 1945.

A decision was reached as to whether or not any given specimen was a potential (if not an actual) flying member of the population. In these two years all individuals found at rest were disturbed by shaking the plants on which they were sitting until they were dislodged. If they flew, they were regarded as potentially active, but if they allowed themselves simply to fall inert, they were regarded as being in a quiescent state at least for the time being and were given a distinctive mark (with celltulose paint).

During these two years, 868 unmarked specimens were caught, and 151 of them were quiescent according to this definition. In all, 40 marked specimens were recaptured, of which 36 were " active " when retaken. Yet 9 of these 36 active recaptures were marked to indicate that they had been quiescent when first found. Moreover, though 4 marked individuals were quiescent when recaptured, one of them had been " active " when it had been originally taken. Thus though a not inconsiderable proportion of the specimens (approaching one-sixth) may be found in a torpid condition, the evidence does not support the view that a significant number of them remains so permanently.

Secondly, it seems probable that females rarely fly for the first 24 hours after emergence (p. 145), and they are certainly the less active at all times. Thus they tend to be in excess among the quiescent captures, as is indicated in table 16, in which the sexes of active and quiescent specimens caught in 1945 are tabulated. Consequently, were our captures restricted to flying specimens which, as already explained, they were not, there would be a tendency to omit a proportion of the females from the calculations.

One general point of importance must be stressed in regard to these suggested sources of error. We believe we have demonstrated that they do not bias our sampling ; but even if it had not been

possible to establish this, they would still be immaterial to our purpose since they would both tend to make our estimate of the total population too small. Now it will be explained in section 6 that the crucial

TABLE 16

The numbers of active and quiescent males and females caught in 1945

	Males	Females	Total
Active	176	133	309
Quiescent . . .	28	35	63
	204	168	372

matter for our conclusions is the lower not the upper limit of the population size, in the sense that our results would be vitiated if we could not demonstrate that this colony has consisted of almost 1000 imagines each year. Provided that our estimates satisfy this requirement, errors which tend to make them too small would only strengthen our conclusions still further. On the other hand, had these suggested sources of error acted in the opposite direction, and tended to indicate that our estimates of the total population were inflated, the validity of our deductions would have been threatened.

Field studies in the area of the Dry Sandford colony might suggest that our estimate of the total number of imagines hatching in any one year (varying between about 1000 to about 6000) is too low, for in their season the nearly full-fed larvæ are extremely abundant. It would be easy to collect 1000 in a few hours and to return the next day and obtain as many with as little trouble. As suggested on p. 144, we have strong reasons to suspect that the chief elimination in the life history of this insect occurs near the end of larval life and in the pupa. The larvæ are almost free from parasitism, nor have we found them attacked by birds. The virus disease which sometimes takes a heavy toll of this insect destroys the larvæ in their last instar, while *Pteromalus puparum*, which is a serious enemy, is purely a pupal parasite. Moreover, moles and mice are extremely common in the habitat, and they may well eat large numbers of pupæ but few, if any, larvæ. Females lay, on the average, between 200 and 300 eggs; consequently, though our estimates suggest that during 1939 to 1945 the total number of imagines emerging in one year has not reached 6000 and has several times been little above 1000, it would not be surprising if the number of well-grown larvæ in this locality was between 50,000 and 100,000 in most of the seasons during which we have been at work.

No simple relation exists between the size of the population per season and the frequency of the *medionigra* gene. Doubtless the fact

that the population numbers are far from stable contributes to, and is itself an expression of, the changes in the direction and intensity of selection which are responsible for the variations in the proportions of the two allelomorphs (pp. 168-71) ; but it would be a situation of perhaps unexpected simplicity if the population-size and gene-frequency showed obvious correlation.

We must at least mention another aspect of our work on *Panaxia dominula*, merely to indicate that it is under investigation though we reserve it for discussion at a later date. From 1939 to 1946 the heterozygotes have on the average occupied 10·8 per cent. of the population, and even after the high value of the first two years their average frequency has been 9·9 per cent. (for 1941-46). Should such a proportion be approximately maintained, or increased, some evolutionary modification of the heterozygous phenotype might conceivably take place fast enough to be detected during a human lifetime, so providing a remarkable opportunity for observing the effects of selection upon the gene-complex of a wild population. We are here assuming the type of selective dominance modification first suggested by Fisher (1928), and this has already been successfully demonstrated in domestic animals by Fisher (1935, 1938), using poultry, and in wild material by Ford (1940) using the Currant Moth *Abraxas grossulariata*, and by Fisher and Holt (1944) using the house mouse. By means of selection experiments, carried out on the heterozygote, which is subject to considerable variability (plate I, figs. 3-6), we are now establishing the inherent possibility of discovering such an effect in the Dry Sandford colony of *Panaxia dominula*. The work is not yet complete, but in seven generations *medionigra* has become relatively more dominant in one line and more recessive in another, the difference being already significant. The variability concerned therefore appears to be of such an order as to encourage us to watch for comparable results in nature, in which case the process of evolution would have been foreshadowed in the laboratory.

(6) THE SIGNIFICANCE OF CHANGES IN GENE-RATIO

Great evolutionary importance has been attached by Sewell Wright (1931, 1932, 1935, 1940) to the fact that small shifts in the gene-ratios of all segregating factors will occur from generation to generation owing to the errors of random sampling in the process by which the gametes available in any one generation are chosen to constitute the next. Such chance deviations will, of course, be greater the smaller the isolated population concerned. Wright believes that such non-adaptive changes in gene-ratio may serve an evolutionary purpose by permitting the occurrence of genotypes harmoniously adapted to their environment in ways not yet explored, and so of opening up new evolutionary possibilities. Consequently, he claims that subdivision into isolated groups of small size is favourable to evolutionary progress, not, as others have thought, through the

variety of environmental conditions to which such colonies are exposed, but, even if the environments were the same for all, through the non-adaptive and casual changes favoured by small population size. Those evolutionists who find it difficult to attach any great evolutionary significance to such chance effects, have urged that the normal segregation of all factors in each generation continually supplies new genotypes selected at random from a number usually much greater than the number in a single generation of even a numerous population, and that the selective increase or decrease of any gene is determined by the totality of the life experience of all these, comprising as they do large numbers of harmonious, or successful, and of inharmonious, or unsuccessful, combinations : that the number of genotypes tried will generally be larger in more numerous than in less numerous populations ; and that the existence of very small and completely isolated populations, such as Wright seems to postulate, will generally be terminated by extinction in a period which must be thought of as short on an evolutionary scale of time.

There is a further possibility which, if established, would cut at the root of the whole theory, namely that populations, large and small, are subjected from generation to generation to selective intensities capable of producing greater fluctuations in gene-ratios than could be ascribed to random sampling. The data obtained on *dominula* are peculiarly well fitted to examine this possibility, since here we have in a succession of generations estimates both of a gene-ratio and of the population-size of the colony. The latter are indeed rough and of unknown precision ; but since of the six years available in only one can the population have been so small as a thousand, and as isolated colonies materially smaller than this must be in constant danger of extinction, it will be worth the while to determine whether the fluctuations in gene-ratio available for eight successive years are so small as to be reasonably ascribable to random sampling in a population of a thousand each year, or whether they show such larger fluctuations as would require the action of selection varying perhaps both in intensity and direction from year to year.

Since the gene frequencies observed vary from about 10 per cent. to less than 5 per cent., the precision of the determinations on a percentage scale would be unequal, even if the numbers of insects had been the same from year to year. For this reason it is desirable to apply the angular transformation, $p = \sin^2\alpha$, for which the information available about the angular measure of gene frequency, α, is proportional to the number of observations. Table 17 shows the number of observations and the angular measures in degrees for the sequence of eight years.

The amount of information for any year will then be independent of α and equal to

$$\left(\frac{\pi}{90}\right)^2 n = \frac{n}{820{\cdot}7016}$$

where n is the number of observations, or twice the number of insects counted.

TABLE 17

	Number n	Angular measure α
1939	446	17°·6497
1940	234	19°·4712
1941	922	15°·1531
1942	410	13°·3938
1943	538	13°·6588
1944	992	12°·2974
1945	744	14°·7144
1946	1972	11°·9108

On the theory to be tested the measures of gene-ratio observed will be connected by a chain of random sampling processes. Each observation, based on a random sample of $\frac{1}{2}n$ insects out of a population of $\frac{1}{2}N$, will diverge from the true value of the population by a difference the variance of which (ignoring the constant) is

$$\frac{1}{n}-\frac{1}{N};$$

while any two successive population values will have a random difference of which the variance is

$$\frac{1}{N}$$

where N is twice the number of the second population. Consequently, choosing the population value of any year as origin we can write down the covariance matrix for samples of given size from eight successive generations. Thus, using the population value for 1942 as origin, we should have the covariance matrix :—

$$\begin{array}{cccccccc}
\frac{1}{n_1}+\frac{2}{N} & \frac{2}{N} & \frac{1}{N} & 0 & 0 & 0 & 0 & 0 \\
\frac{2}{N} & \frac{1}{n_2}+\frac{1}{N} & \frac{1}{N} & 0 & 0 & 0 & 0 & 0 \\
\frac{1}{N} & \frac{1}{N} & \frac{1}{n_3} & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & \frac{1}{n_4}-\frac{1}{N} & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & \frac{1}{n_5} & \frac{1}{N} & \frac{1}{N} & \frac{1}{N} \\
0 & 0 & 0 & 0 & \frac{1}{N} & \frac{1}{n_6}+\frac{1}{N} & \frac{2}{N} & \frac{2}{N} \\
0 & 0 & 0 & 0 & \frac{1}{N} & \frac{2}{N} & \frac{1}{n_7}+\frac{2}{N} & \frac{3}{N} \\
0 & 0 & 0 & 0 & \frac{1}{N} & \frac{2}{N} & \frac{3}{N} & \frac{1}{n_8}+\frac{3}{N}
\end{array}$$

Inserting in this expression the eight values of n from table 15, with N equal constantly to 2000, the arithmetical values of the covariance matrix giving the random simultaneous deviations of the eight observations from any chosen value for the 1942 population are shown in table 18. This has been simplified by inserting a factor of 10,000.

TABLE 18

Numerical values of covariance matrix

32·4215	10	5	0	0	0	0	0
10	47·7350	5	0	0	0	0	0
5	5	10·8460	10	0	0	0	0
0	0	0	19·3902	0	0	0	0
0	0	0	0	18·5874	5	5	5
0	0	0	0	5	15·0806	10	10
0	0	0	0	5	10	23·4409	15
0	0	0	0	5	10	15	20·0710

The matrix shown in table 18 is next inverted to provide the information matrix of these eight observations. This is shown in table 19, in which the factor of 10,000 has been removed. Associated

TABLE 19

Numerical values of information matrix

347·0642	−58·7863	−132·8960	0	0	0	0	0
−58·7863	230·0762	−78·9645	0	0	0	0	0
−132·8960	−78·9645	1019·6665	0	0	0	0	0
0	0	0	515·7244	0	0	0	0
0	0	0	0	599·3877	−140·6958	−32·8447	−54·6714
0	0	0	0	−140·6958	1077·2624	−208·0013	−346·2272
0	0	0	0	−32·8447	−208·0013	863·9748	−533·8740
0	0	0	0	−54·6714	−346·2272	−533·8740	1083·3411

with table 19 we have marginal totals formed by adding the entries in each row, and a second series derived from each row by multiplying each entry by the angular measure of gene frequency and adding. These two series are shown in table 20. If a_{ij} is a typical entry in the information matrix, these are

$$A_i = \sum_j a_{ij}$$

$$T_i = \sum_j a_{ij}\alpha_j$$

The ratio of the totals from table 20 is $\bar{\alpha} = 14°{\cdot}23245176$, and the heterogeneity of the observations on the hypothesis considered is found from the expression $\Sigma a_i T_i - \bar{\alpha}\Sigma T_i$, or numerically 17207·2297. Since angles have been measured in degrees this is divided by 820·7016 to obtain the value of χ^2 for the seven degrees of freedom

among eight independent observations. We find, therefore, $\chi^2 = 20{\cdot}8064$, a value which demonstrates very significant heterogeneity, since the 1 per cent. value is only 18·475. Not once in a hundred

TABLE 20

Margin and weighted margin of information matrix

	A	*T*
1939	155·3819	2967·1528
1940	92·3254	2245·7422
1941	807·8060	11568·0003
1942	515·7244	6907·5095
1943	371·1758	5322·2540
1944	382·3381	4141·3336
1945	89·2548	3347·5100
1946	148·5685	43·3833
	2567·5749	36542·8859

random trials, therefore, would differences in gene-ratio so great as those observed be exhibited by observations of random samples of the sizes used, from a population of 1000 in each generation, in which reproduction was at random, and in which, therefore, changes in gene-ratio are ascribable to random survival only. Still less could they have arisen in a larger population.

The conclusion that natural populations in general, like that to which this study is devoted, are affected by selective action varying from time to time in direction and intensity, and of sufficient magnitude to cause fluctuating variation in all gene-ratios, is in good accordance with other studies of observable frequencies in wild populations.* We do not think, however, that it has been sufficiently emphasised that this fact is fatal to the theory which ascribes particular evolutionary importance to such fluctuations in gene-ratio as may occur by chance in very small isolated populations. Evidently, however large a population might be, its gene-ratios will fluctuate in the same manner and to approximately the same extent as those of the smallest isolated population which can be expected to persist in nature.

(7) SUMMARY

1. The spread of a gene in an isolated colony of the moth *Panaxia dominula* L. (family Arctiidæ) has been studied from 1939 to 1946, and compared with its frequency prior to 1929.

2. The habits of the moth and the nature of the colony are described.

3. Specimens heterozygous for the gene in question are distinguishable, as var. *medionigra*, from the other two genotypes, but they

* For instance, considerable seasonal changes affecting gene-ratios or inversion-frequencies have been demonstrated by Dobzhansky (1943) and Timoféeff-Ressovsky (1940).

resemble more closely the normal form *dominula* than the rare homozygote var. *bimacula*.

4. Prior to 1929, the frequency of the *medionigra* gene in this colony was not more than 1·2 per cent. In 1939 it had reached 9·2 per cent., and in 1940 11·1 per cent. (20·5 per cent. of the population being heterozygotes). From 1941 to 1946 it has fluctuated round a mean of 5·2 per cent., showing no tendency to return to the high value of 1939-40 or to the low one observed previous to 1929.

5. From 1941 onwards, the absolute numbers of this population have been estimated by the method of marking, release and recapture, which is described. The data so obtained are recorded by means of trellis diagrams.

6. New methods have been devised for analysing these results.

7. Our findings are consistent with a constant death-rate, such that 16 per cent. of the population are eliminated daily, giving an average expectation of life of 6·25 days.

8. Tables 7 to 11 give estimates of the absolute numbers flying on each day of collecting.

9. There is no indication that in nature the forms *medionigra* and *bimacula* emerge earlier or later than the normal *dominula*.

10. Estimates have been obtained year by year for the total emergence during the whole season. This did not fall below a minimum of 1000 (in 1943) nor rise above a maximum of 6000 to 8000 (in 1946). During 1944 (total emergence 5000 to 6000) as many as 3000 moths might have been flying at the time of maximum abundance.

11. It has been suggested that two sources of error might have affected our estimates of the absolute numbers: that a significant proportion of the specimens may remain quiescent throughout their lives, and that the females rarely fly for the first 24 hours after emergence. It has been shown that such considerations do not influence our results. Moreover, both would tend to make the estimates too low; so that they would not in any event have vitiated our conclusions, which require that the total yearly population of imagines should not fall appreciably *below* 1000.

12. A simple direct relation between population-size and the frequency of the *medionigra* gene is scarcely to be expected, and has not been found.

13. Breeding experiments conducted in the laboratory have shown that the expression of the *medionigra* gene can be modified by artificial selection. Watch is being kept for any corresponding effect in nature under the influence of natural selection. It is possible that this may be detected if the present frequency of heterozygotes in the colony is maintained or increased.

14. An angular transformation of gene-frequency, for which the information available becomes proportional to the number of observations, is used to test whether the observed yearly fluctuations

Panaxia dominula L.

FIG. 1.—Typical form, the common homozygote (*dominula*).

FIG. 2.—*Ibid.* One of the marked specimens used. The three marks, in different colours and positions, each indicate a date of capture and release.

FIGS. 3-6.—Heterozygotes (var. *medionigra* Cockayne), showing less and more extreme forms.

FIGS. 7-8.—The rare homozygote (var. *bimacula* Cockayne), showing less and more extreme forms.

in gene-ratio are small enough reasonably to be ascribed to random sampling in the reproduction of a population of 1000 each year.

15. It is, in fact, found that the observed fluctuations in gene-ratio are much greater than could be ascribed to random survival only. Fluctuations in natural selection (affecting large and small populations equally) must therefore be responsible for them. The possibility that random fluctuations in populations much smaller than 1000 could be of evolutionary importance is improbable in view of the frequency with which such small isolated populations must be terminated by extinction within periods which must be extremely short from an evolutionary point of view.

16. Thus our analysis, the first in which the relative parts played by random survival and selection in a wild population can be tested, does not support the view that chance fluctuations in gene-ratios, such as may occur in very small isolated populations, can be of any significance in evolution.

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SPECIES CROSSES IN *ANTIRRHINUM*

I. GENETIC ISOLATION OF THE SPECIES *MAJUS, GLUTINOSUM* AND *ORONTIUM*

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Received 17.iv.47

(I) THE SPECIES

A. majus and *A. glutinosum* both belong to the section *Antirrhinastrum* of the genus; yet they differ markedly in both morphology and distribution. *A. majus* is too well known in cultivation to require description here. Its cultivation is world wide in temperate countries, and even its natural distribution seems to be fairly extensive. Bentham and Hooker assign the species to the Mediterranean region generally. Its distribution in Spain and Portugal has been followed more closely by Baur (1932), who described the species as occupying the whole of the northern and western portion of the peninsular.

The distribution of *A. glutinosum* has been given both by Baur (1932) and in the *Botanical Magazine* (1893). These authorities agree in regarding the species as confined to a small mountain area, the Sierra Nevada region of south-eastern Spain. A closely similar species is found, similarly distributed, in the Pyrenees.

Although *A. majus* is highly variable in cultivation, it shows a number of general differences from *A. glutinosum*. A full description of the latter will be found in the *Botanical Magazine* (1893). It has a more prostrate habit than *A. majus*, and its leaves are smaller, rounder and hairier. Flower shape is not widely different between the species, but the flowers of *A. glutinosum* are smaller in size. They are also preponderantly ivory in colour, though a little anthocyanin is found in them. The young floral buds have a pale red-buff appearance; but in the adult flower the red anthocyanin appears only as a number of upright lines on the front or inner part of the upper lobe of the corolla.

The strain of *A. glutinosum* used in these experiments was obtained from the Royal Botanic Gardens, Kew, in 1942. It agrees well with the description and illustration in the *Botanical Magazine*, except that in this country it does not seem to be perennial as claimed in that description. This claim is qualified, however, by the remark that protection by glass is needed if winter survival is to be assured. The failure of our plants to over-winter may thus not be out of harmony with the description.

Ten inbred lines of *A. majus* have been used in the experiments. Their flower colours are as follows :—

1 line—Ivory anthoxanthin with full red anthocyanin.
1 line—Ivory anthoxanthin with full shell anthocyanin (pelargonidin derivative).
2 lines—Ivory anthoxanthin with spots and stripes of red anthocyanin, due to an evermutating gene.
2 lines—Ivory anthoxanthin with reduced anthocyanin, appearing as a flush of red colour.
3 lines—Yellow anthoxanthin. No anthocyanin.
1 line—Ivory anthoxanthin. No anthocyanin.

The absence or reduction of anthocyanin in seven of the last eight strains depends on the multiple allelomorph series, termed the *pal* series by Kuckuck and Schick (1930), one member of which is the evermutating gene. The genes causing loss of anthocyanin in one of the yellow strains have not been isolated and classified.

The first nine strains were used for crossing with *A. glutinosum*. The last was used in a cross with a third species, *A. orontium*. This is a small-flowered species which grows as a weed in parts of Great Britain. It is said by Bentham and Hooker to have originated in Southern Europe, but now to be widespread as a weed of cultivation over all Europe and Central Asia. It is of upright habit, with narrow leaves and long sepals. In colour, its corolla is magenta-red. This strain of *A. orontium* was also obtained from Kew in 1942.

(2) NATURAL CROSSES

It is not clear whether *A. majus* and *A. glutinosum* have opportunities for intercrossing as wild species. Baur's map suggests that they do not, as their distributions are shown as not overlapping. The possibility of intercrossing when the species are artificially intermingled was, however, tested by an experiment in 1944.

One hundred plants of *A. glutinosum* and one hundred of *A. majus* were grown in a single outdoor plot of $10' \times 20'$, the plants being spaced at $1'$ each way. The hundred plants of *majus* were further divided into fifty from one of the strains with a mosaic of red on ivory flower colour resulting from the evermutating gene, and fifty from one of the strains with a flush of anthocyanin on ivory flower colour. The two hundred plants were assigned to places at random within the plot. Mortality was somewhat high among the plants of *A. glutinosum*, and the experiment ended with only seventy-four out of the hundred plants of this species, as compared with all fifty mosaic and forty-nine of the fifty flushed plants of *A. majus*.

Pollination was allowed to take place naturally by insects, chiefly bees, among the plants of the plot. At the end of the season, seed was collected from thirty-three plants of each of the three types. This

was sown in 1945, and twelve plants were grown from each of the ninety-nine mothers. With a few exceptions these plants survived to flowering, when their origin, whether by intra- or inter-specific pollination could be recorded. The results are set out in table 1.

TABLE 1

Natural cross-pollination of A. majus *and* A. glutinosum

Mother	Within species			Between species	Aberrant	Unclassifiable	Total
	Within variety	Between varieties	Uncertain				
majus mosaic	269	29	22	4	2	67	393
majus flushed	337	41	...	0	1	18	397
glutinosum .	335	...	...	7+3?	17	38	400

Percentages of species crossing

Mother	Percentage species crosses	
	Certain	Maximum
majus mosaic . .	1·2	18·2
majus flushed . .	0·0	4·5
majus pooled . .	0·6	11·3
glutinosum . .	2·9	12·5

The species hybrids were readily distinguishable by their having flowers with a uniform coloration of somewhat pale anthocyanin on ivory. In *A. glutinosum* intra-specific pollinations would all give the same result; but in *A. majus* a further classification was possible of the intra-specific pollinations. Crosses between the two varieties, mosaic and flushed, were detectable by their being mosaic on flush.

Certain aberrant types of plant appeared in the progenies. Two of these plants were obviously strays from the families of flushed mothers into a family of a mosaic mother. A third, also in a family from a *majus* mother, seemed to be a mutant of some kind, and the remaining seventeen were scattered throughout the *A. glutinosum* progenies. These seventeen all showed some signs of having genes from *A. majus*, but they were equally clearly not F_1s. It is believed that they represent crosses of the *A. glutinosum* plants with a group of species F_1s, F_2s and backcrosses which was growing about 400′ away from the natural pollination test plot.

A further apparently aberrant class, including thirty-seven plants, was found in the progenies from *A. majus* mothers. These had flowers with uniform anthocyanin coloration; yet from the intensity of this

colour and from their general morphology, they were obviously not species hybrids. They were in fact true *A. majus*, the product of gametes from the mosaic strain carrying the mutated allelomorph of the ever-sporting gene. Such gametes are known to be produced by the ever-sporting strain. Where the plants appeared in the progeny of flushed mothers, they must have been from the inter-varietal cross within the species. When in the progeny of mosaic mothers their origin is uncertain ; they could be from either mosaic or flushed fathers, for the presence or absence of flush could not be detected in them.

A total of 123 plants out of 1190 were not in flower at the time of classification and so were unclassifiable. They appear as such in table 1. Taking only the plants which were classified and which were not aberrant, the percentages of species crosses in the progenies of the various types of mother are as given in the column marked " Certain " in the lower part of table 1. These are all low, the highest being under 3 per cent. It might be objected, however, that the unclassified plants, or a disproportionately high proportion of them, might be species hybrids. Assuming them all to be hybrids, the percentages of species crosses listed in the column marked " Maximum " are found. Even these figures never exceed 20 per cent. ; and it is almost certain that they are gross overestimates.

Species hybrids are generally recognisable in the progenies of *A. glutinosum* by their foliage. The thirty-eight plants of the progenies which did not flower appeared not to carry an excessive proportion of hybrids as judged in this way. No similar classification is possible in the progenies of *A. majus* mothers. There was, however, no correlation between proportion of hybrids and number classified in these progenies, such as would be expected were the hybrids later flowering and so tending to be left unclassified disproportionately often. In fact, observations on species hybrids over a period of several years suggest that they tend to flower earlier than their *A. majus* parent.

Thus we have no reason to expect excessive proportions of hybrids in the unclassified plants. The estimates in the " Certain " column are probably reasonably accurate, and species crossing probably did not exceed 3 or 4 per cent. of the pollinations.

Crossing occurs therefore between the species, but it is rare ; rarer in fact than varietal crosses within *A. majus*, as shown by the natural crossing of the mosaic and flushed strains ; and rarer too than intra-specific crossing with *A. glutinosum* which, as we shall see, is virtually self-incompatible under outdoor conditions. There must be some means of genetic isolation between the species.

(3) ARTIFICIAL CROSSES

Genetic isolation of the two species could be brought about in either of two ways. It could be due to a relative failure of the pollen

of one species to achieve fertilisation after reaching stigmata of the second species, *i.e.* to interspecific incompatibility such as is known, for example, in *Petunia* ; or it could be due to a relative failure of the pollen of one species to reach stigmata of the other. The former possibility was tested in three ways : by comparison of the success of inter- and intra-specific pollinations as judged by set of capsules ; by the segregation of marker genes in the progeny of the species hybrid ; and by the success of inter- and intra-specific pollination in competition after simultaneous double pollination.

The first test was carried out in 1943, using plants both out-of-doors and in the greenhouse. Self-pollinations, intercrosses of plants of the same species and intercrosses of plants of different species were made and their successes compared. The third species, *A. orontium*, was included in the outdoor test. The results are given in table 2.

TABLE 2

Pollination tests

Cross	Outside pollinations			Inside pollinations		
	Made	Successful	Percentage	Made	Successful	Percentage
majus selfed	133	82	*62*	13	12	*92*
× *majus*	15	14	*93*	1	1	*100*
× *glutinosum*	96	69	*72*	71	66	*93*
× *orontium*	116	1*	*1*	...	...	...
glutinosum selfed	27	1	*4*	13	5	*38*
× *glutinosum*	20	7	*35*	3	2	*67*
× *majus*	32	15	*47*	16	11	*69*
× *orontium*	12	0	*0*	...	...	...
orontium selfed	8	7	*88*	...	...	...
× *orontium*	13	9	*69*	...	...	...
× *majus*	15	0	*0*	...	...	...
× *glutinosum*	17	2†	*12*	...	...	...

* This seed failed to germinate, but germination had been obtained earlier from a single successful cross.

† This seed failed to germinate.

Outdoor crosses of pairs of *A. majus* plants were highly successful. Self-pollinations were less so outside, though indoors these selfs were virtually as successful as the outside crosses. The lines used were mainly the inbreds already described. We might expect that, as a result of the effects of inbreeding, self-pollination within such lines would be less often successful than would cross-pollination between them, particularly under outdoor conditions.

The set of capsules on *A. majus* mothers after pollination by *A. glutinosum* was greater than with self-pollination out-of-doors and much the same as with it in the greenhouse. Inter-specific crossing was somewhat less successful than intra-specific crossing out-of-doors,

but not significantly so. There was, in fact, nothing to suggest that *A. glutinosum* pollen was relatively unsuccessful in *A. majus* stigmata.

The pollinations on to *A. glutinosum* mothers tell the same story. In this species, however, self-pollination, even under glass, was relatively unsuccessful, and out-of-doors the species behaves as virtually self-incompatible. Crosses within the species were less often successful than those within *A. majus*, but this may well have been due to the use of inter-incompatible plants as parents in a proportion of cases. Crosses with *A. majus* were actually more often successful than the crosses with other plants of *A. glutinosum*.

In contrast to these results are those obtained when *A. orontium* was used as a parent. This species is easily self-pollinated, and crosses can be made within the species fairly readily. The flower is small and emasculation damage may well account for the small reduction in success of crosses as compared with selfs. *A. orontium* has, however, never set seed with pollen of *A. majus* and only rarely with that of *A. glutinosum*. The reciprocal cross has given no capsules with *A. glutinosum* and only a few with *A. majus*. In only one case, in the year before the test under consideration, has any of the seed from a cross of *A. majus* by *A. orontium* germinated to give hybrid plants. Clearly, although there is no barrier to successful functioning of pollen once pollination has been achieved between *A. glutinosum* and *A. majus*, there is a strong barrier to the success of pollen in the crosses between these two species on the one hand and *A. orontium* on the other.

For the second test of the success of inter-specific pollination, F_1s from crosses of *A. majus* and *A. glutinosum*, made in 1942, were in 1943 backcrossed both as males and females to the parental species. In these backcrosses segregation can be observed of genes whose differences contribute to the distinction in flower colour of the two species. When such an F_1 is used as the female parent the segregation reflects only the relative viabilities of the various gametic combinations which are produced by the hybrid. When the F_1 is used as male, however, pollen competition also affects the segregation observed in the backcross progeny, as the following consideration will show.

Where the parent species differ in a gene or genes causing inter-specific incompatibility, pollen from their hybrid will vary, some of it carrying the allelomorph from one species, and some from the other. When this segregating pollen is used on the stigma of either parental species, grains carrying the allelomorph from that same species will grow better, and achieve fertilisation more often, than grains carrying the allelomorph from the other species. Where such incompatibility genes are linked with marker genes affecting flower colour, the segregation of the latter will be correspondingly upset in the male backcross. There will be more of the progeny resembling the species to which the backcross is being made when the F_1 is used as male than when it is used as female. Such differences in segregation therefore afford a test of the presence of any pollen growth genes by

which the species may be characteristically distinguished. This test has already revealed isolation genes acting by affecting pollen growth in the species cross between *Petunia axillaris* and *P. violacea* (Mather and Edwardes, 1943).

Crosses of *A. glutinosum* on to the various inbred lines of *A. majus*, on backcrossing to *A. majus*, revealed the action of six different genes. One gene affects the intensity of the anthocyanin pigmentation. A number of allelomorphs from *A. majus* were involved, namely the evermutating gene, another leading to absence of anthocyanin and at least two others giving reduced anthocyanin production (one of which gave the flushed type used in the test of natural pollination). In all cases, *A. glutinosum* contributed an allelomorph giving full anthocyanin pigmentation. The segregations of this series of allelomorphs in backcrosses to *A. majus* are pooled in the first section of table 3 (*a*).

The second section of that table shows the joint segregation of two complimentary genes for anthocyanin productions observed in the cross of a yellow anthocyaninless form of *A. majus* by *A. glutinosum*. One of these genes may be the same as that described above. The third section shows the segregation for a gene affecting anthoxanthin colour, the ivory allelomorph having been introduced from *A. majus*. The fourth presents data on the segregation for the " delilah " colour patterns introduced by another inbred line of *A. majus*, and the fifth section gives corresponding information about the segregation of a gene causing shell or coral anthocyanin colour in *A. majus*.

The data are pooled in the last section of table 3 (*a*), all the individuals carrying the various allelomorphs from *A. majus* being summed above and those carrying allelomorphs from *A. glutinosum* summed below.

In three of the sections the male backcross shows an excess of individuals carrying the allelomorph from *A. majus* as compared with the female backcross ; but in the other two the reverse is the case. The pooled data also show a slight excess of *A. majus* allelomorphs in the male backcross. In no case, however, whether of individual segregation or of the pooled data, is the difference between male and female backcross segregation significant when tested by the χ^2 appropriate to a contingency table.

Table 3 (*b*) gives corresponding data for three segregations in the backcrosses of these same F_1's to *A. glutinosum*. The segregation for reduced as opposed to full anthocyanin appears to depend on one gene ; but those for absence as opposed to presence of anthocyanin, and for ivory as opposed to pale yellow anthoxanthin, are more complex, probably depending on several genes. The data have nevertheless been pooled, as in the *A. majus* backcrosses, to give a joint test. Again the excess of types having the allelomorph from *A. glutinosum* sometimes shows in the male and sometimes in the female backcross. No significant difference is present in any part of the table.

In contrast to these results are those of table 3 (*c*). The hybrid between *A. majus* and *A. orontium* was backcrossed reciprocally to *A. majus*, the backcross to *A. orontium* failing both ways. Segregation

TABLE 3

Segregation in backcrosses of A. majus × A. glutinosum *to*

(*a*) A. majus (*b*) A. glutinosum

Character	Allele	Hybrid as		Total	Allele	Hybrid as		Total
		Father	Mother			Father	Mother	
Anthocyanin :								
Reduced	*m*	54	48	102	*g*	124	134	258
Full	*g*	68	76	144	*m*	114	130	244
Anthocyanin :								
Absent	*m*	39	34	73	*g*	60	68	128
Present	*g*	12	13	25	*m*	24	26	50
Anthoxanthin :								
Ivory	*m*	11	14	25	*g*	68	77	145
Pale yellow	*g*	18	10	28	*m*	37	43	80
Anthocyanin distribution :								
Delilah	*m*	30	20	50	...	...	...	...
Normal	*g*	19	29	48	...	...	...	...
Anthocyanin :								
Shell	*m*	13	17	30	...	...	...	...
Red	*g*	12	5	17	...	...	...	...
Pooled Data	*m*	147	133	280	*g*	252	279	531
	g	129	133	262	*m*	175	199	374

m indicates the class receiving the *majus* allelomorph(s) and *g* the *glutinosum* allelomorph(s) from the hybrid.

(*c*) *Segregation in backcrosses of* A. majus × A. orontium *to* A. majus

Allelomorph	Hybrid as		Total
	Father	Mother	
majus	24	48	72
orontium	10	67	77

was observed for one gene determining anthocyanin, from *A. orontium*, as opposed to no anthocyanin, from *A. majus*. The female backcross shows an insignificant excess of the allelomorph from *A. orontium* over the expected 0·5. The male backcross shows an excess, and a significant excess, of the allelomorph from *A. majus*. A contingency

table χ^2 brings out the great significance of the excess of individuals with the *A. majus* allelomorph as the male backcross over the female. Selective pollen growth is clearly revealed by these data.

The picture is therefore the same as that shown by the first test. No evidence was found of inter-specific incompatibility genes tending to isolate *A. majus* and *A. glutinosum* by lowering the relative success of pollen on the style of the foreign species ; but there is clear evidence of at least one such gene distinguishing *A. majus* and *A. orontium*.

The third test was confined to *A. majus* and *A. glutinosum*. In 1945 stigmata of flowers of each species were pollinated simultaneously

TABLE 4

Pollination of A. majus *and of* A. glutinosum *with mixtures of pollen of the two species*

Mother	Progeny		
	A. majus	Hybrid	*A. glutinosum*
A. majus :			
Family 1	5	4	...
,, 2	3	0	...
,, 3	12	85	1*
,, 4	13	9	...
,, 5	1	9	...
,, 6	30	85	...
,, 7	2	6	...
,, 8	36	28	...
,, 9	14	2	...
,, 10	5	10	...
,, 11	...	4	...
Total	121	242	1*
A. glutinosum :			
Family 1	...	34	16
,, 2	5*	19	11
,, 3	...	10	25
,, 4	...	2	21
,, 5	...	...	17
Total	5*	65	90

* Presumably these plants are strays.

with pollen of both species. Sometimes this was done by applying first *A. majus* pollen immediately followed by *A. glutinosum* pollen. In other cases the reverse order was adopted. In a third set of pollinations the two types of pollen were mixed as well as possible in a small dish, and the mixture then applied to the stigma. Whenever *A. glutinosum* stigmata were pollinated, pollen was taken from a different plant of this species for use in conjunction with that from *A. majus*, in order to reduce the risk of complication from the self-incompatibility of

A. glutinosum. Seed was collected from the capsules to which mixed pollinations gave rise and progenies grown from it in 1945.

These progenies were classified by means of the flower colour into the two classes obtained from functioning of the two kinds of pollen. As already reported (Mather, 1946), the progenies from the *A. glutinosum* mothers were also earlier classified on the basis of seedling appearance, but this was not possible where *A. majus* was used as mother, because the hybrid closely resembles this species vegetatively. A number of plants died between the two classifications of the progenies from *A. glutinosum*, so that although the results agree in overall appearance they differ a little in numerical detail.

The results from classification on flower-colour are shown in table 4. Both maternal and hybrid plants were obtained from the mixed pollinations on to both *A. majus* and *A. glutinosum* mothers. The frequencies are somewhat irregular from family to family, as is to be expected with double pollinations, and there is an overall excess of plants from *A. glutinosum* pollen when used on both types of mother. The excess is, however, smaller with *A. glutinosum* mothers than with *A. majus*. It is not to be traced to any isolating mechanism between the species. The small number of aberrant plants in a few of the families cannot detract from the strength of our conclusion. In the experiment as a whole, hybrid plants were as common as those of the two specific types. There is no barrier to the success of the foreign pollen, once the pollination has been made artificially. All three tests agree, therefore, in showing that there is no inter-specific incompatibility between *A. majus* and *A. glutinosum*.

(4) BEE BEHAVIOUR

Since no isolating mechanism can be found between *A. majus* and *A. glutinosum* once pollination has been achieved, we must look for the isolation in the failure or near-failure of inter-specific pollination under natural conditions. It appears that the pollinating insects, mainly if not entirely bees, do not move pollen from flowers of the one species to those of the other, even though they often transfer pollen from flower to flower and plant to plant within one species.

This conclusion was tested by observations on the bees working over the mixed plot used for the test of natural inter-specific pollination in 1944. The number of observations of this kind was not so large as could have been desired.* So far as they went, however, the direct observations of bee behaviour confirmed the conclusion reached from the genetical observation of the plants.

Individual bees were seen at work on both species, but no bee was ever seen to enter flowers on a plant of one after entering those on a plant of the other. The bees would move freely from flower to flower and from plant to plant of one species, even flying through an

* Owing to circumstances adversely affecting the conduct of genetical experiments in London during that summer.

inflorescence of the other kind in so doing ; but they never paused to enter flowers of the species with which they were not concerned. Up to twenty visits were seen to be made by a single bee without any change in the species visited, the bee then soaring away from the pollination plot, presumably to return to the hive.

Since some species crosses were observed in the progeny of the plants from this plot, it must be assumed that the bees, or other insects, moved from one species to the other in a small proportion of cases. This change-over need not, however, have occurred during one working period : it may have been brought about by a bee returning to the opposite species after a soaring flight of the kind made when visiting the hive. This type of flight has been considered to lead to the rare long distance contamination seen between varieties of the same species (Bateman, 1947). The production of the aberrant progeny from *A. glutinosum* by crosses with the derivatives of the species hybrid growing some 400 feet from the pollination plot shows that such long distance contamination was in fact occurring.

These aberrant progeny of *A. glutinosum* were also instructive in another way. It is known that bees can distinguish certain colour differences, including some to which man is not sensitive (Frisch, 1914), and it might be supposed that the flower colour differences between the strains of *A. majus* and *A. glutinosum*, used in the natural pollination plot, were responsible for the distinction which the bees made between them. Some of the aberrant progeny of *A. glutinosum* had, however, coloured flowers, so suggesting that the bees would transfer pollen from plants with coloured flowers to others with white ones. This was confirmed a generation later when nearly one-half of the progeny obtained from a plant of *A. glutinosum* had coloured flowers. It therefore seems that the colour difference cannot be responsible in this case for the differential behaviour of the bees.

Thus bees will not commonly transfer pollen from the one species to the other, even though the plants are growing side by side. They will transfer pollen from F_1 or later derivatives of the species cross to *A. glutinosum* at least, even though the plants are separated and have unlike flower colours. Flower colour is not the deciding factor. The precise nature of the phenotypic difference between the species which leads the bees to discriminate between them remains to be found out. Further investigation will also be needed to find out how much genetic variation exists within a species, of the kind which would lead to differential bee behaviour, and so would represent the material from which this new type of isolating mechanism could be built up.

(5) SUMMARY

When grown together in a single plot, and allowed to pollinate naturally, *A. majus* and *A. glutinosum* show less than 3 per cent. inter-crossing.

Pollination by artificial means is as easy between these species as within them. Furthermore, when stigmata of either species were pollinated simultaneously with pollen from the two, hybrids were produced as commonly as maternal types.

Segregation of marker genes in backcrosses of the inter-specific hybrids reveals no trace of genes determining an inter-specific incompatibility reaction.

There is therefore no mechanism isolating *A. majus* and *A. glutinosum* once pollen has been successfully transferred to a stigma of the opposite type.

The species *A. majus* and *A. orontium*, on the other hand, are isolated by an inter-specific incompatibility reaction, as is shown both by the difficulty of making the hybrid and by the disturbed segregation of a marker gene in the backcross of the hybrid to *A. majus*.

Observations of bee behaviour show that the mechanism isolating *A. majus* and *A. glutinosum* is to be found in the adherence of the bees to one or other species when working over a mixed stand. Pollen transference from one species to the other was never seen to occur.

Bees have been shown to transfer pollen from species hybrids of F_1, or a derived generation, having coloured flowers, to *A. glutinosum* with its predominantly white flowers. Some difference other than in flower colour therefore seems to be responsible for the discrimination which the bees make between *A. majus* and *A. glutinosum*.

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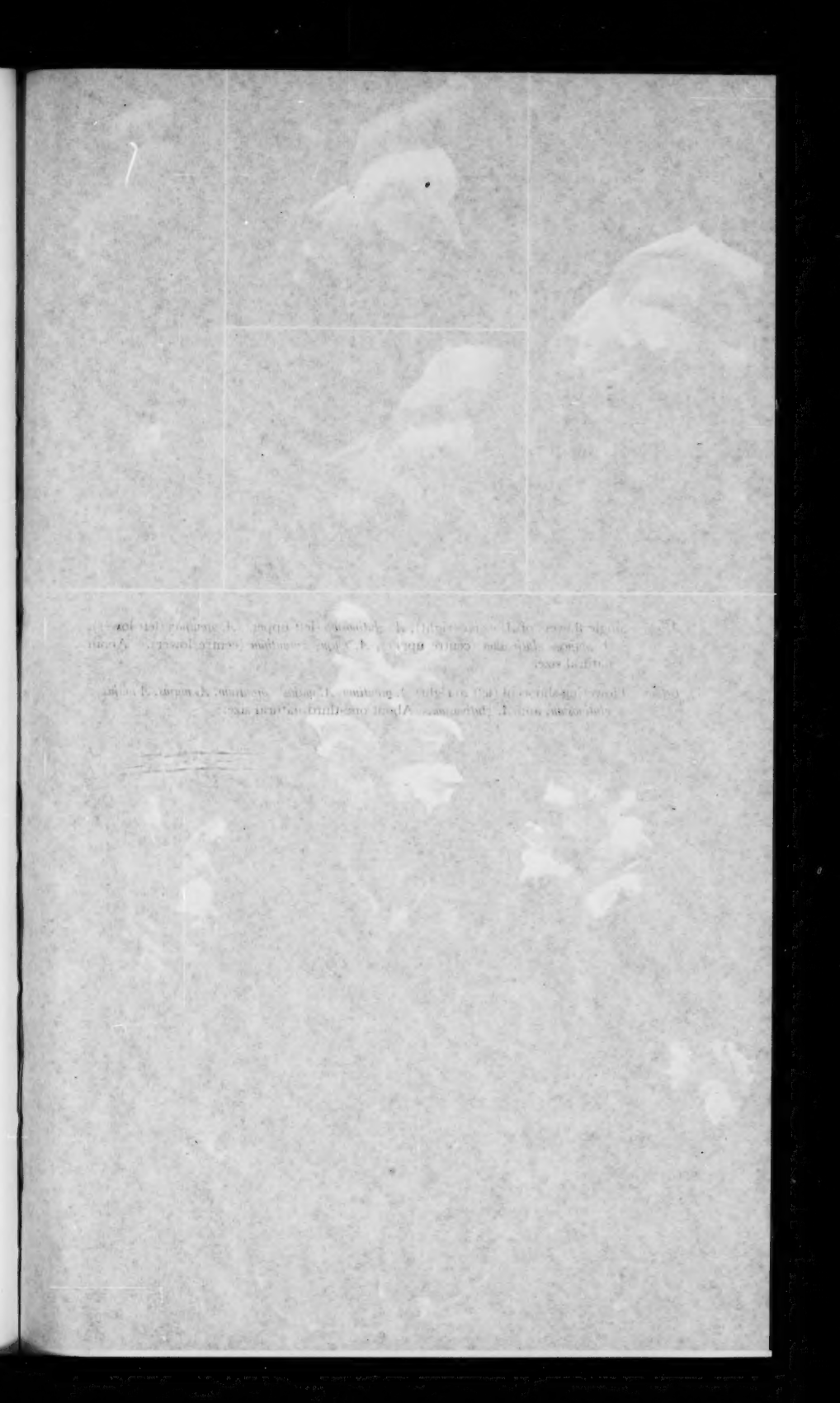
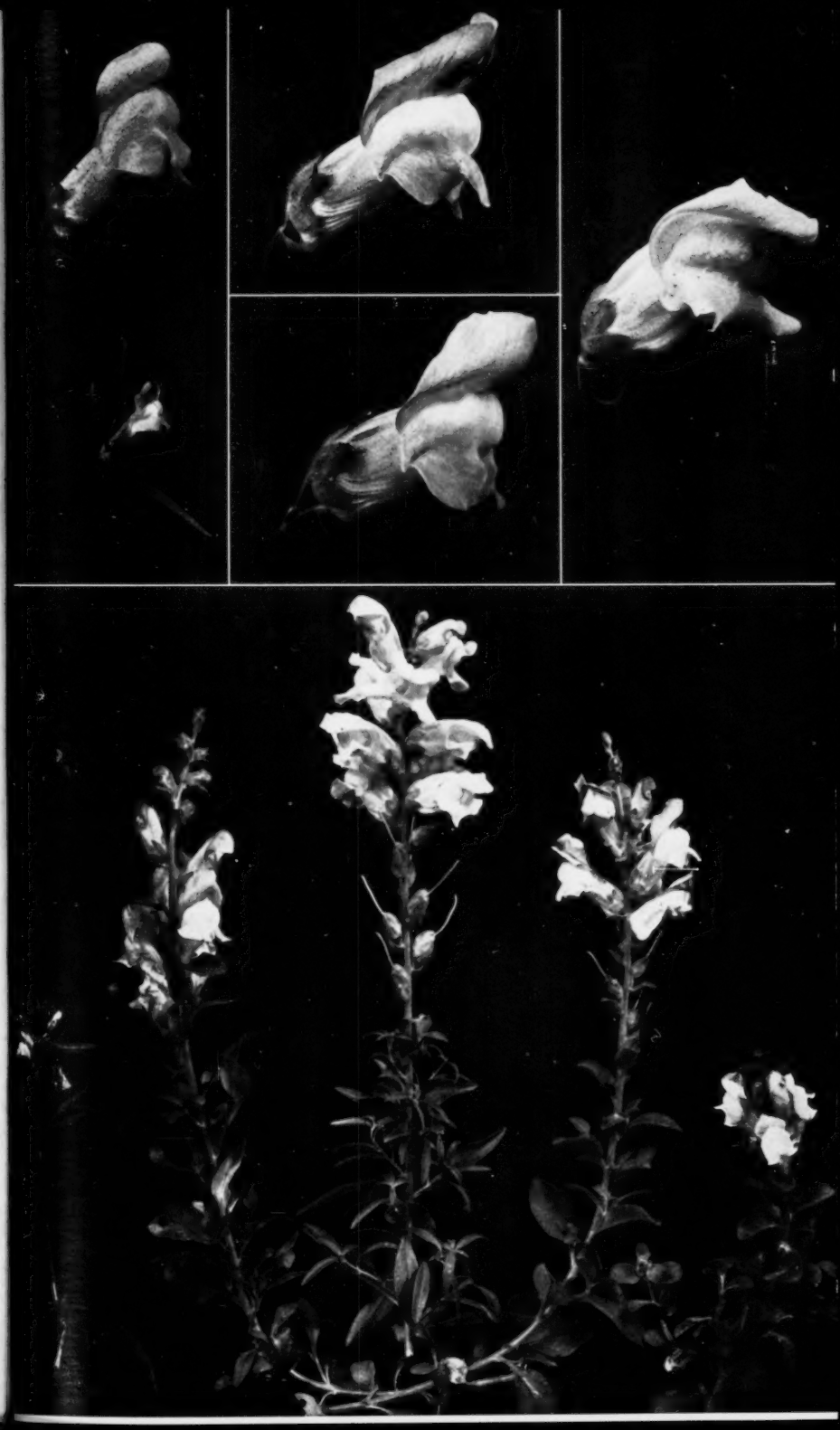


Above. Single flowers of *A. majus* (right), *A. glutinosum* (left upper), *A. orontium* (left lower), *A. majus* × *glutinosum* (centre upper), *A. majus* × *orontium* (centre lower). About natural size.

Below. Flowering shoots of (left to right) *A. orontium*, *A. majus* × *orontium*, *A. majus*, *A majus* × *glutinosum*, and *A. glutinosum*. About one-third natural size.



THE CHEMICAL BREAKAGE OF CHROMOSOMES

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CONTENTS

	PAGE
1. Different ways of Breaking Chromosomes .	187
2. Introducing Mustard Gas	189
3. Methods of Treatment	190
4. Effects on Cell Development	191
5. Effects on the Chromosomes	
(i) Qualitative	194
(ii) Quantitative : Mitosis . . .	206
(iii) „ : Meiosis . . .	210
6. Chemical, Radiation and Spontaneous Effects .	211
7. The Target Theory	215
8. Summary	217
References	218

(1) DIFFERENT WAYS OF BREAKING CHROMOSOMES

INJURIES to chromosomes and genes fall into two classes which we describe as spontaneous and induced. The spontaneous effects are due to errors or abnormalities in the reaction of the genotype to the environment. They probably cover the whole gamut of possibilities. At one extreme no environment will remedy the mutafacient effect of the abnormal genotype. Here we have Beadle's " sticky " maize. At the other extreme no genotype will remedy the effect of the abnormal environment. Here we have sister-reunion of chromatids due to non-division of the end-gene, a regular property of aged pollen. Since temperature is the chief environmental variable concerned, and since low temperature will produce by nucleic acid starvation the same sister-reunion, we see that the distinction between spontaneous errors and induced ones is purely a matter of the exactness of our control which is continually increasing.

An important section of effects could never be described as spontaneous. They arise by three agencies :—

(i) *Ionising radiations*, such as X-rays, α and β particles and neutrons (Lea, 1946).

(ii) *Molecular excitation* with ultra-violet light (Stadler, 1941 ; Mackenzie and Muller, 1940 ; Hollaender, 1946).

(iii) *Chemical treatments*: the substances which act as nuclear mutafacients, or gene and chromosome breakers,* are of many kinds. So far we know that the following can induce gene or chromosome mutations or both, without killing:—

Potassium thiocyanate (Stubbe, 1940).
Ethylurethane (Oehlkers, 1943).
Sulphur and nitrogen mustards (Auerbach, 1943; Koller, 1943; Reports to the Ministry of Supply; Horowitz *et al.*, 1946).
Sulphonamides (Chevais and Thomas, 1943).
Allyl isothiocyanite (Auerbach and Robson, 1944).
Phenol (Hadorn and Niggli, 1946).

Of these different treatments the most detailed account of the effects is that given for the action of X-rays on mitosis in plants (Darlington and La Cour, 1945). The effects may be classified in the light of recent knowledge as of three kinds (*cf.* Darlington, 1942, 1947).

Centromere effects. These consist of breakage at or within the centromere of one or both chromatids. Such breakage may happen either before metaphase (when it may be followed by reunions or at least fluid nucleic acid attachments), or at anaphase under the strain of movement. Distinct from this breakage is misdivision of the centromere such as takes place spontaneously at meiosis and leads to the formation of isochromosomes by reunion of the chromatids within the centromere.

Nucleic acid effects. These are sometimes associated with fluid, sticky, or unpolymerised chromosome surfaces, due to too great a charge, and sometimes with the opposite condition of starvation. Both go with errors of spiralisation, and also of reproduction, especially in end-genes, and especially at meiosis.

Similar errors occur spontaneously at pre-meiotic divisions, and in degenerating nurse cells. The nucleic acid excess leads to an immediate hastening of mitosis but, if it is carried too far, a reaction follows and mitosis is stopped.

Fibre breakage effects. The ordinary chromosome thread is broken when the treatment occurs in the resting stage. Most of this stage is before the division or reproduction of the thread, and the result of

* A different kind of mutafacient effect is that found in the *carcinogenic* compounds. Malignancy which depends on a capacity for the unrestricted proliferation of cells can be induced by various reagents, especially polycyclic hydrocarbons such as methylcholanthrene and benzpyrene. These reagents may alter cell behaviour at the place of application or at distant sites; they may also affect all tissues and organs or only some, no doubt with a specific kind of activity. The transformation to malignancy is thought in the first place to be *cytoplasmic* both on genetic grounds (Darlington, 1944; Haddow, 1944; van Potter, 1945; and Woods and Du Buy, 1945) and on cytological grounds (Koller, 1947*b*).

Nuclear mutafacients on the other hand start reactions which quickly invade the nucleus and attack its permanent structure which is unharmed by the carcinogens. Of course, chemical agents might be found—methylcholanthrene (Strong, 1945) is perhaps of such a type—to induce permanent changes in both the nucleus and the cytoplasm, acting as nuclear and cytoplasmic mutafacients at the same time (*cf.* M. Demerec, 1947: *Nature*, *159*, 604).

breakage therefore appears at the ensuing metaphase as a break in both chromatids (B″) and gives two types of fragment with and without centromeres (C_1″ and C_0″). The broken ends of these fragments can undergo reunion (R″) in new combinations to give dicentric chromosomes (C_2″) or rings (C_0″ or C_1″). Their constituent chromatids may also undergo separate reunion after reproduction (R′) to give configurations which visibly reveal inversions and interchanges at the ensuing metaphase. In addition, at the moment of splitting, an error may occur in the reproduction of the end-gene (or of any nucleic-acid-starved gene) giving sister-reunion (SR). Finally, after reproduction, breakage is of chromatids (B′) and reunion likewise (R′).

Prolonged low intensity X-ray doses have enabled us to separate these three groups of effect. Experiments by Koller (1946 and unpub.), using doses of 0·1 r, 0·25 r and 0·5 r per minute up to 200 r, on *Tradescantia* pollen grains, have shown the centromere effect clearly for the first time. They have also shown three-armed or *triradial* figures, due to the union of B″ ends with B′ ends, in a particularly high frequency. And finally, they have shown a new *concentration effect*. Particular cells, or even chromosomes, in a sample suffer non-random damage. They are completely shattered. Not merely *minute* fragments but *subminutes* are formed.

Spontaneous errors are of most of the various types shown by induced errors ; but the types appear separately in different instances. It seems that the spontaneous effects are more specific. Since they do not include the concentration effect arising from prolonged treatment perhaps their specificity is due to a more specific time of action. In this regard it is notable that B″ occurs without B′ in *Tulipa fragrans* and that centric breakage occurs at mitosis in *Tradescantia* but only at meiosis in *Tulipa orphanidea* (Darlington and Upcott, 1941).

We may expect these considerations to help, and to be helped by, the study of any new results of chemical action.

(2) INTRODUCING MUSTARD GAS

At the outbreak of the late war, research was taken up in various universities of Great Britain, Canada and, later on, the United States, in order to find antidotes for " vesicants " such as lewisite and mustard gas. At that time already some foundations had been laid. Cashmore and McCombie (1923) had already concluded that these vesicants reacted directly with the protein constituents of the cell (*cf.* Hartwell, 1946). Later it had been shown, *in vitro*, that oxygen consumption and glycolysis are reduced in cells after mustard gas treatment (Berenblum *et al.*, 1936). The new work indicated that mustard gas inactivates essential cellular enzymes concerned with phosphate transfer to and from adenylic compounds (*cf.* Gilman and Philips, 1946).

For a time enzyme inactivation was considered to be the primary

mechanism by which mustard gas affected the cell. Later, however, it was found that very weak mustard gas did not affect cell respiration, glycolysis, or those enzyme functions which depend on the phosphokinases. Yet it produced fundamental changes in the activity of the living cell. Canadian and American research also suggested that when the cells recovered after weak treatment they showed extensive damage to the nucleus. By using genetical methods, Auerbach (1943) was able to show that sulphur mustard ($\beta\beta'$-dichlorodiethylsulphide) induced gene changes. She obtained over 700 sex-linked lethals and many visible recessive and dominant mutations in *Drosophila*. Breeding tests also indicated rearrangements of chromosomes which were verified from the salivary glands of the progeny (Slizynski, 1943).

Three important points emerge from this work as summarised recently by Auerbach, Robson and Carr (1947). First, the mutations are of the same kind as those induced by X-rays. Secondly, the same treatment as gives 9 per cent. of sex-linked lethals gives only 0·5 per cent. of II-III interchanges instead of 6 per cent. as X-rays would, as though there were less rejoining of breaks. Thirdly, more than twice as many mosaics are produced as with comparable X-ray doses, and these mosaics have progeny mosaic for the same mutation as the parent, indicating a special faculty of producing unstable genes or unstable chromosomes.

In view of the importance of these discoveries, a detailed study of the direct effects of mustard gas on the chromosomes was undertaken. The raw data of the present observations were communicated to the Ministry of Supply in 1942-43, by Koller and others, in a report which was at the time confidential.

(3) METHODS OF TREATMENT

In the first set of experiments in the summer of 1942, root-tips, an inch long, of the onion, *Allium cepa*, were dipped in pure fluid mustard gas for 1-30 minutes, washed in running water for 10 minutes, and fixed 30 minutes, as well as 12, 24 and 48 hours, after treatment. In all but the first fixation the effects on the resting and dividing cells were too drastic to be recorded even qualitatively. They had all been killed.

In the next series of experiments (1943), germinating seeds of the onion were exposed in a bell-jar to the vapour of mustard gas. Five minutes' exposure led to chromosome fragmentation, interfered with the spindle and, consequently, the anaphase movement. From these tests and those carried out on *Drosophila*, the use of mustard gas in vapour form seemed to be a promising method. It was used in all the further work.

In the main experiments pollen mother cells and pollen grains of the diploid *Tradescantia bracteata* were treated. A satisfactory apparatus

was devised by Dr J. M. Robson. The inflorescences were kept in a small chamber through which an air current was passing at a constant rate. Vaporised mustard gas was released into the air current at intervals of 15 seconds. The mustard gas was used, either in pure, concentrated form, or diluted in cyclohexane in the ratio of 1 : 10 or 1 : 100. The dose and time of exposure could be controlled with some accuracy. The amount of damage varied in successive experiments owing chiefly no doubt to differences in the depth of penetration of the vapour. There was no means of measuring the different amounts of the poison which might be absorbed by cells during the different stages of their development or at different depths in the anther. The qualitative conclusions, as in Auerbach's experiments, and those derived from the internal statistics of the work, are unaffected by these difficulties.

Anthers were also exposed to *di-* and *trichloroethyl amines* or nitrogen mustards. The effects obtained were similar to those induced by the sulphide. Such quantitative differences as occurred might be due to the different vapour pressures of the three substances.

Anthers were fixed in 3 : 1 acetic alcohol 6, 12, 24, 48, 72 and 120 hours after treatment and stained with acetic lacmoid or Feulgen's basic fuchsin. The timing is to be taken only comparatively since the temperatures varied over the range 19°-24° C. between night and day.

The method of treatment is given in the legends of the figures. Thus " 1 : 100/2 min./24 hrs." means that the cell was treated with diluted mustard gas (1 : 100), exposed for 2 minutes and fixed 24 hours after treatment.

(4) EFFECTS ON CELL DEVELOPMENT

General. A gross toxic effect is quickly induced in cells at all stages of development if they are exposed to heavy doses. Clumping of metaphase chromosomes, clotting or contraction of the prophase and resting nuclei, vacuole-formation in the cytoplasm, were seen in *Allium* root-tips 30 minutes after exposure to the vapour of pure mustard gas. The cells did not recover, and as already noted, the whole treated portion of the root died.

In treatments of pollen grains with all degrees of dilution we can distinguish two types of nuclear change so severe as to preclude the study of breakage. The first is *clotting* ; a physiological wrecking, a clumping of prophase or metaphase chromosomes into a large, irregular-shaped, deeply stained body (plate II, fig. 7). From this damage pollen grains probably do not recover. The second is *shattering* ; a mechanical wrecking, an extreme breakage of chromosomes (plate II, fig. 8). The many large and small nuclei formed after shattering cannot be expected to survive.

The proportion of pollen grains wrecked in these two ways after

treatment with various concentrations of mustard gas are given in table 1 and fig. 1. The proportions of damaged pollen mother cells

TABLE 1 (*cf.* fig. 1)

Percentage frequencies of cells wrecked (bold face) and merely broken (roman) as seen in mitosis after treatment with mustard gas for 2 minutes. Samples of 300 pollen grains. Temperature 19°–24°C.

Strength	Times in hours after treatment			
	6	12	24	48
1 : 100	10·0	12·6	10·0	69·3
	...	5·0	12·6	9·3
1 : 10	11·3	16·0	19·6	45·3
	...	14·0	45·3	34·3
Pure	44·0	31·3	20·3	30·0
	...	23·6	53·0	58·6

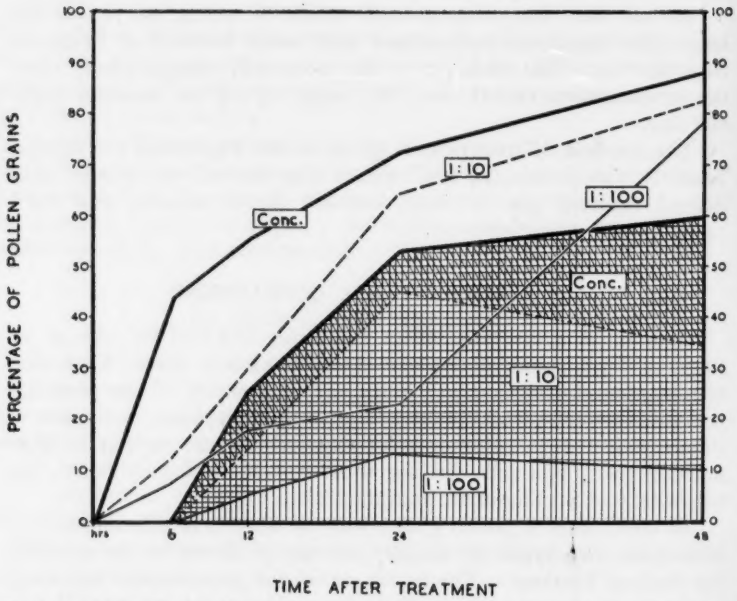

FIG. 1.—The frequencies of affected pollen grains from 6 to 48 hours after treatment with pure and diluted mustard gas. The proportion of pollen grains which are excessively damaged are shown by hatched areas.

and pollen grains increases with concentration of dose. They also increase with time up to 48 hours.

In other words the effect of the poison is cumulative. Dilute

mustard gas has the advantage for experimental use that the frequency of recordably broken cells reaches its maximum more gradually and is always higher than that of wrecked cells.

TABLE 2 (*cf.* fig. 2)

Percentage of metaphases (M) and anaphases (A) in samples of 200 pollen grains after treatment with mustard gas for 2 minutes

Strength		Times after treatment in hours				
		Zero (control)	6	12	24	48
1 : 100	M	6·5	10·5	11·5	12·5	7·5
	A	*4·0*	*5·0*	*19·5*	*8·5*	*4·5*
	T	10·5	15·5	31·0	21·0	12·0
Conc.	M	,,	19·5	9·5	13·5	5·5
	A	,,	*3·5*	*25·5*	*6·5*	*7·5*
	T	,,	23·0	35·0	20·0	13·0

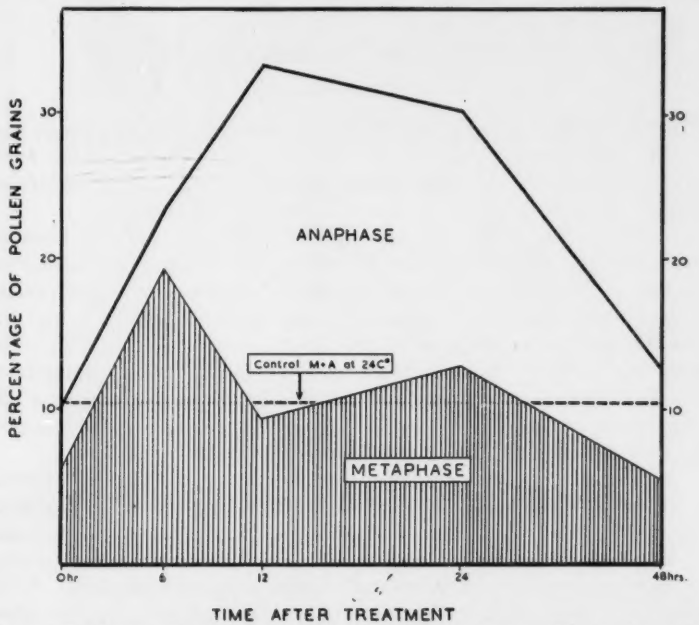

FIG. 2.—The frequencies of cells at anaphase and at metaphase at different times after treatment with pure mustard gas.

Mitosis. The frequencies of metaphases and anaphases are of course changed after treatment with mustard gas (table 2 and fig. 2).

At 24° C. the normal level of joint metaphase and anaphase frequency is about 10·5 per cent. in anthers of *Tradescantia* with 20-25 per cent. of binucleate cells. Development is only slightly less affected after treatment with dilute than with pure mustard gas. Both increase the proportion of pollen grains in metaphase and in anaphase 6 hours after treatment. This change is probably due in part to the slowing down of both these stages (as suggested by defects in spindle formation) and in part to the hastening of prophase (as suggested by the comparable X-ray experiments).

Synchronisation. Cells in the same, or nearly the same, stage of development are differently affected by treatment according, no doubt, to depth in the anther. In pollen mother-cells 24 hours after treatment the normal synchronisation within dyads is consequently upset. After 72 hours this failure of synchronisation extends even between mother-cells. Some cells were found in the first metaphase while others were already undergoing the second division. Delayed metaphases showed the severest breakage. Similar behaviour was seen in pollen grains : the division of a small proportion is delayed 3-4 days, *i.e.* until the generative nucleus of the sister grains is in process of differentiation.

(5) EFFECTS ON THE CHROMOSOMES

(*i*) *Qualitative*

The effects of the minimum doses on the pollen grain mitosis and on the preceding meiosis are similar in a general way. They may be grouped under the same three headings as the X-ray effects as follows (*cf.* table 3).

A. *Centromere effects.* These appear in the first samples both in the prophase nucleus of mitosis and in the interphase nucleus of meiosis. They take the form of occasional apparent breakage leading to separation of sister chromatids at the centromere (figs. 3 and 9). But this effect can be no more than a cross-wise stretching since (in the early samples) no consequences have been seen at metaphase. The first signs of error at metaphase are a lengthwise stretching of the centromere and a failure of congression on the plate which is followed by various defects in the anaphase movement (Koller, 1947*a*).

Later, centromeres misdivide in mitosis as well as in meiosis. Also single chromatids can break off a centromere at anaphase. Later still, this breakage is sometimes found at metaphase in mitosis. It seems always to be followed by reunion with another centromere, or end, or even union with the middle of a chromosome. Such reunion is due presumably to a nucleic acid and not a protein fibre connection, since it does not seem to survive in any anaphase configuration. In the later samples anaphase movement becomes more defective ; chromosomes lag and extra nuclei appear at telophase ; wall-formation may fail in the pollen mother cell.

TABLE 3

TABLE 3

Summary of effects at mitosis *and* meiosis *after 2 minutes of 1 : 100 treatment*

	Times	6 hrs. (fig. 3)	12 hrs. (3d, 4)	24 hrs. (fig. 5)	48 (6, 7, 8) 72, 120 hrs.
Mitosis	Centromeres	Defects shown by separation at *prophase*, stretching at *metaphase*, errors of congression on the plate and of movement to the poles	All defects continue. Mis-division begins	All defects continue. Centric breakage and reunion begin	All primary errors diminish, secondary effects increase (*e.g.* micronuclei)
	Nucleic acid	Excess, hence stickiness and over-coiling	Continued, hence errors in gene reproduction at ends and in middles and hence bridges and fragments at *anaphase*	Continued but with irregular starvation as well	Chromatid attraction may lapse prematurely. Other effects diminish
	Protein fibre	No intercalary breakage	No intercalary breakage	B and R begin with minutes and subminutes, hence fragments at *metaphase* and bridges at *anaphase*	Continued
	Times	6-12 hrs. (fig. 9)	24 hrs. (fig. 10)	48 hrs. (fig. 11)	72 hrs. (fig. 12)
Meiosis *	Centromeres	Same as mitosis	Same as mitosis	No centric breakage but some union, and misdivision	Wall-formation often fails
	Nucleic acid	Also irregular coiling	Same as mitosis, but some starvation begins ; also non-coiling at M I	Non-synchronisation of cells at M II	Non-synchronisation increases
	Protein fibre	Same as mitosis	Same as mitosis	Subminutes less frequent. All R is within chromosomes (owing to *pachytene* B″) hence no change visible until A I	R between chromosomes (owing to *pre-pachytene* B″) gives multivalents, fragments and unequal bivalents at M I

* Compared with mitosis at the corresponding times in the same column.

B. *Nucleic acid effects.* The first samples show an excessive nucleic acid charge associated as usual with stickiness and often with irregular coiling so that the spiral becomes externally obvious. Later these errors increase and, in consequence, at meiosis there is some breakdown

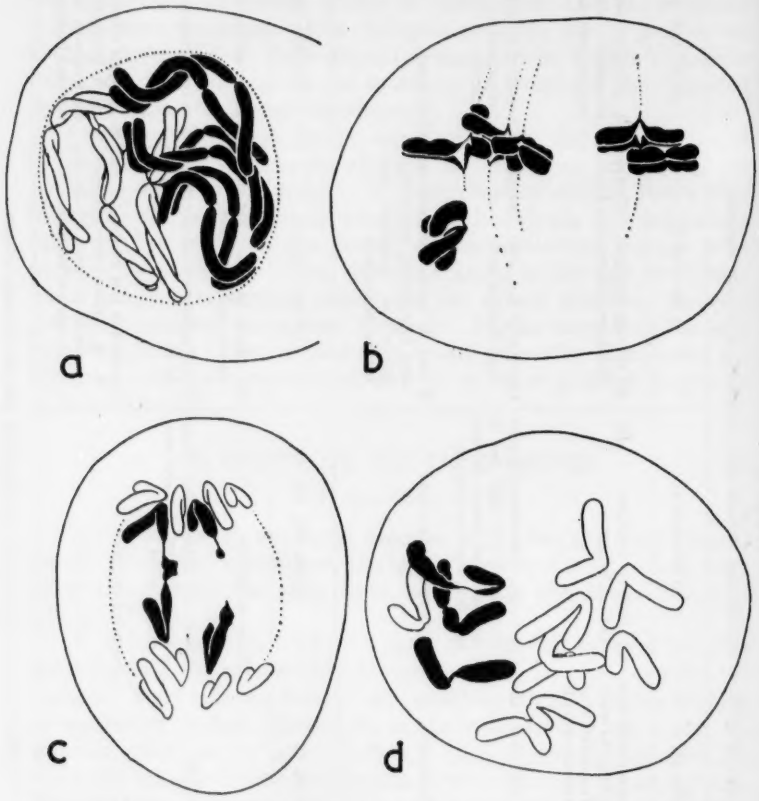

FIG. 3.—(*a*) Prophase of mitosis in treated pollen grains showing separation of centromeres and stickiness at the centromeres and ends. (1 : 10/2 min./6 hrs.)
(*b*) Metaphase : supercontraction of chromosomes, lack of synchronisation in the division of centromeres in different chromosomes and unorientated chromosome in which the centromere is delayed. (1 : 10/2 min./6 hrs.)
(*c*) Anaphase : defective reproduction of chromosomes, local non-spiralisation, and possibly a broken bridge. (1 : 100/2 min./6 hrs.)
(*d*) Anaphase without spindle : some of the chromosomes show stretching at the centromere. (1 : 100/2 min./12 hrs.)

All drawings are magnified 1900 diameters.

of major coiling such as results from heat shock (Darlington and La Cour, 1947). The opposite condition, nucleic acid starvation, also develops sporadically but this is more characteristic of high doses.

Two secondary effects are referable to nucleic acid derangement. One is the failure of synchronisation already noted at the second metaphase of meiosis. The other, seen especially at mitosis, is the failure of gene reproduction and consequently of chromatid separation. This failure, as after cold treatment, is both terminal and intercalary. The one gives rise to bridges, the other to fragments as well.

A premature failure of chromatid attraction at metaphase of mitosis (giving it the appearance of a second meiotic metaphase) is perhaps due to an error of control by the centromere of nucleic acid charges.

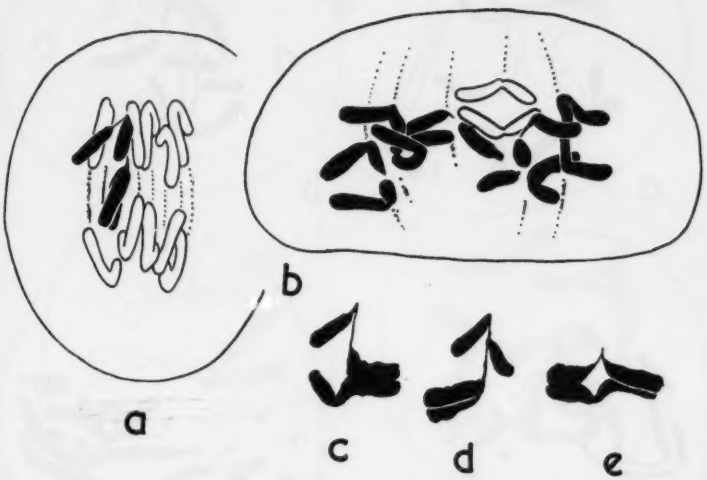

FIG. 4.—(*a*) and (*b*) Anaphase : stretching and misdivision of the centromere. Dicentric chromatids in (*b*) are formed by non-division of an unbroken end. (1 : 100/2 min./12 hrs.)

(*c*), (*d*), and (*e*).—Failure of chromatid reproduction accompanied by stretching and misdivision of the centromere. (1 : 100/2 min./12 hrs.)

C. *Protein fibre effects.* These consist in the breakage of the gene-string otherwise than at the centromere. The breakage takes place inside the resting inter-mitotic nucleus. In consequence its effects are not seen at metaphase of mitosis until 24 hours after treatment.

Apart from large fragments, minutes are formed. They are common at mitosis, rare at meiosis. The two types found with X-ray breakage are recognisable and with similar frequencies, namely (i) those lying unattached and assumed to have undergone R″ to form rings, and (ii) those attached to major chromosomes by *prophase reunion* and assumed to have undergone R′ at one end and perhaps SR at the other (plate II, figs. 7*e*, 8*a*). The highest frequency of these two types is at 72 hours.

In addition to the ordinary minutes there is a class of smaller

subminutes. These are especially abundant 24 hours after treatment and gradually fade away in the later samples. The fact that the subminutes never appear to be double we attribute to their small size. If attractions are a function of mass and repulsions of surface, the attractions would cease to be effective below a certain size (*cf.* Darlington, 1937 ; fig. 139).

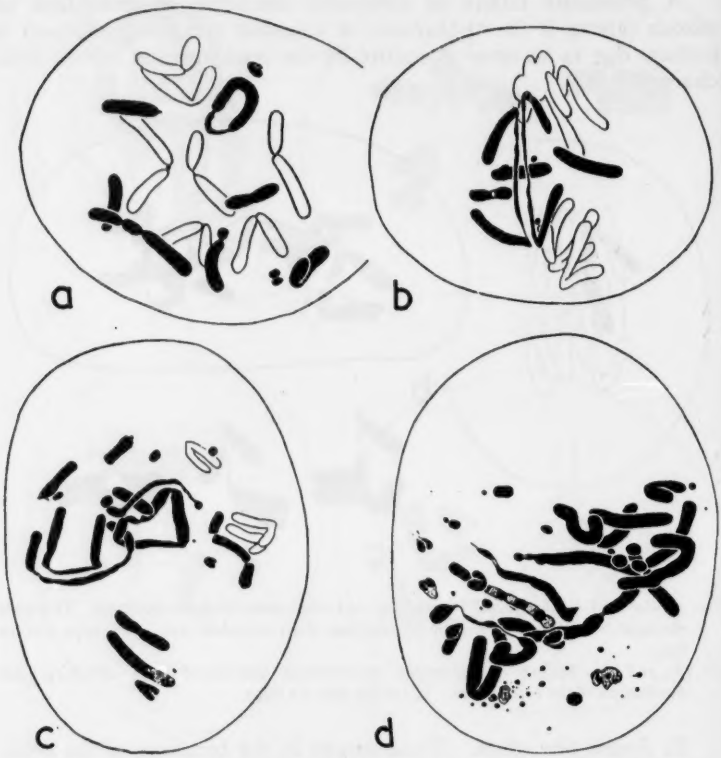

FIG. 5.—(*a*), (*b*), and (*c*). Anaphases with defective spindles, with dicentric chromosomes, centromere breaks of chromatids, bridges, acentric and minute fragments. (*a*) Shows a subspiralised segment not under tension. (1 : 10/3 min./24 hrs.)

(*d*) Extreme chromosome fragmentation or shattering. Many of the fragments are very small, " subminutes." Some chromosome regions and fragments are starved of nucleic acid. (Pure mustard gas/2 min./24 hrs.)

Subminutes may be free globules of nucleic acid, but since they survive at telophase and since they are relatively infrequent at meiosis, where the minutes are also infrequent, we prefer to suppose that they are true chromosome fragments (containing protein fibre).

In X-rayed mitosis a proportion of ends produced by B″ reunite with the opposite ends produced by a single B′ to give triradial figures.

These are most significant. They show that B″, formed before the reproduction of the gene-string, can remain open and unhealed long enough to reunite after its reproduction. Still more significant are certain triradials which arise where *unbroken ends* (no fragments being present) have behaved like B″ ends. These show that unbroken ends can undergo union in special circumstances. We already know that

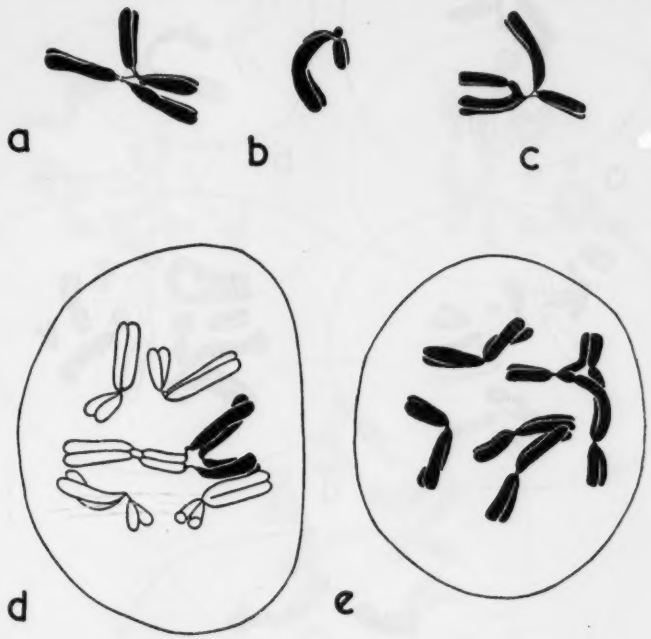

FIG. 6.—(*a*), (*b*), and (*c*). Various types of centric reunion.
(*d*) Metaphase : one chromsome shows centric chromatid break with terminal reunion. (1 : 100/2 min./48 hrs.)
(*e*) Metaphase with one B′ followed by union of the broken ends with two unbroken chromatid ends to give the triradial figure. The union has not established joint spiralisation and so must be a prophase reunion. (*Cf.* plate II, fig. 11). (1 : 100/4 min./48 hrs.)

they can undergo SR, as broken ends do, when the nucleic acid metabolism is upset. It seems, therefore, that such an upset arising from irradiation is responsible for the fusability by R′ of unbroken ends.

Now we find that triradials from both causes are much more frequent after mustard gas treatment than after short intense X-raying (tables 4 and 5, figs. 7e, 8b and 8c, broken ends ; and 6e, unbroken ends). The reasons for this are presumably twofold. First, B′ and B″ are formed directly one after the other in the same cell, whereas with short X-raying they occur together only when one chromatid

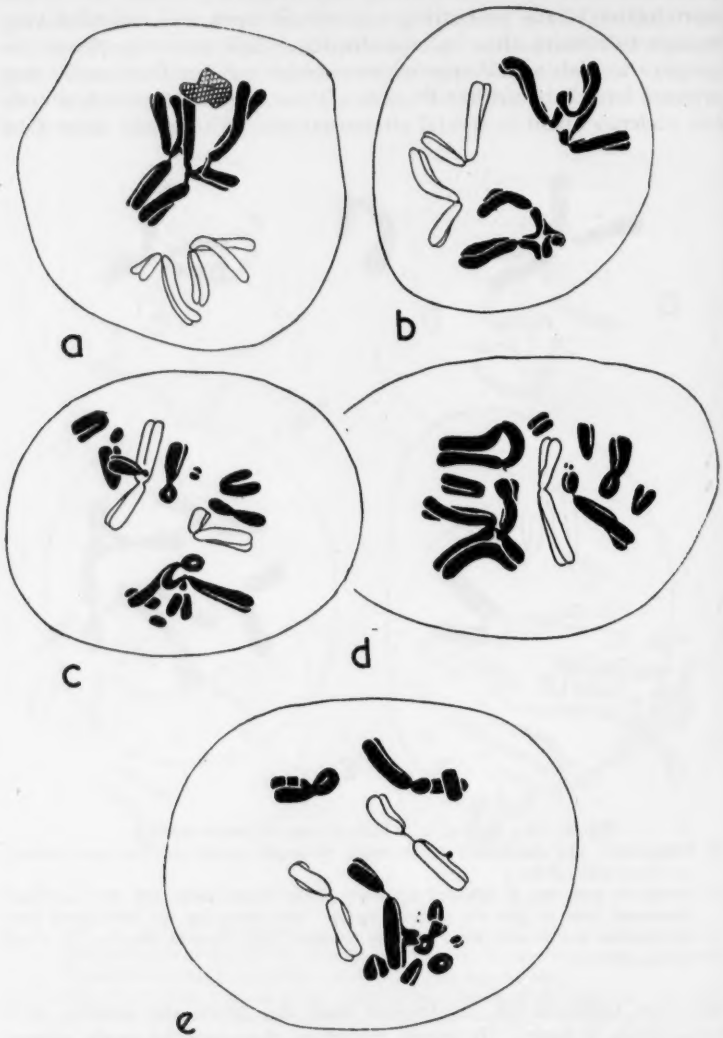

FIG. 7.—Metaphases in pollen grains showing the combination of centric and intercalary breakage and reunion. (1 : 100/2 min./48 hrs.)

(*a*) A collapsed chromosome and multiple centric reunion.
(*b*) Two chromatid interchanges (2B′+2R′).
(*c*) B′ and B″ followed by SR and R″.
(*d*) The foregoing together with premature dissolution of a centromere.
(*e*) Prophase and triradial figures, stretched centromeres, etc.

from a B″ undergoes restitution leaving the other open as a spurious B′ along with genuine B″s. Secondly, the nucleic acid upset can have taken effect on the unbroken ends by the time the chromatid with which they are to unite is broken. The upset by itself provides twelve joinable ends all ready to fuse with any true breaks that may later turn up. Both these differences arise from the prolongation of treatment and not from the kind of treatment.

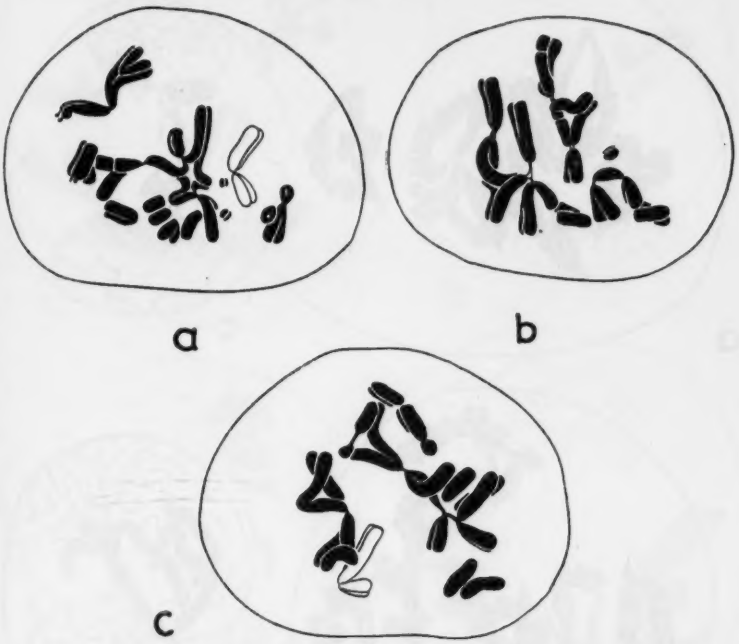

Fig. 8.—Metaphases in pollen grains showing triradials. (1 : 100/2 min./72 hrs.)
(*a*) B″ followed by R″, SR and R′.
(*b*) and (*c*). B′ and B″ followed by R′. Some centric unions.

In meiosis the effects of fragmentation do not appear for an even longer time than in mitosis and then in two stages. First, we find fragmentation at anaphase only and with no abnormalities at metaphase. Later, we find fragments, multivalents and unequal bivalents, at metaphase. Now a sequence of three stages in the conditions of breakage and reunion would be expected at meiosis :

(i) *post-pachytene* ; B′ only ; no reunion except by crossing-over ; no free fragments until the lapse of chromatid attraction at anaphase ;

(ii) *pachytene* ; B″ with no reunion between chromosomes but free fragments at metaphase ;

(iii) *pre-pachytene* ; B″ with free reunion giving multivalents visible at metaphase.

The end terms of this sequence are clearly distinguishable as indeed they also are with X-ray treatments of the same material (La Cour, 1946).

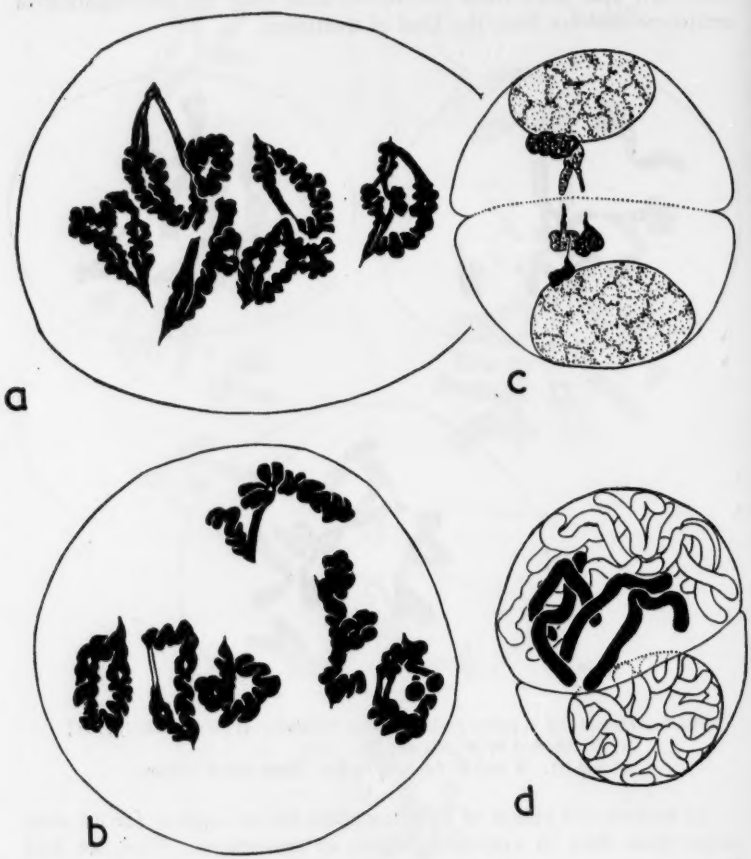

FIG. 9.—Treated pollen mother cells.
(*a*) Metaphase I with irregular coiling. (Pure/2 min./6 hrs.)
(*b*) The same. (1 : 100/2 min./12 hrs.)
(*c*) Lagging chromosomes, excluded from the interphase nuclei. (1 : 100/2 min./12 hrs.)
(*d*) Apparent precocious division of centromeres during prophase of second division. (1 : 100/2 min./12 hrs.)

Thus breakage takes place at prophase of meiosis when the stretched thread between chromomeres is naked and unprotected, although not at prophase of mitosis when the chromosomes are uniformly clothed with nucleic acid.

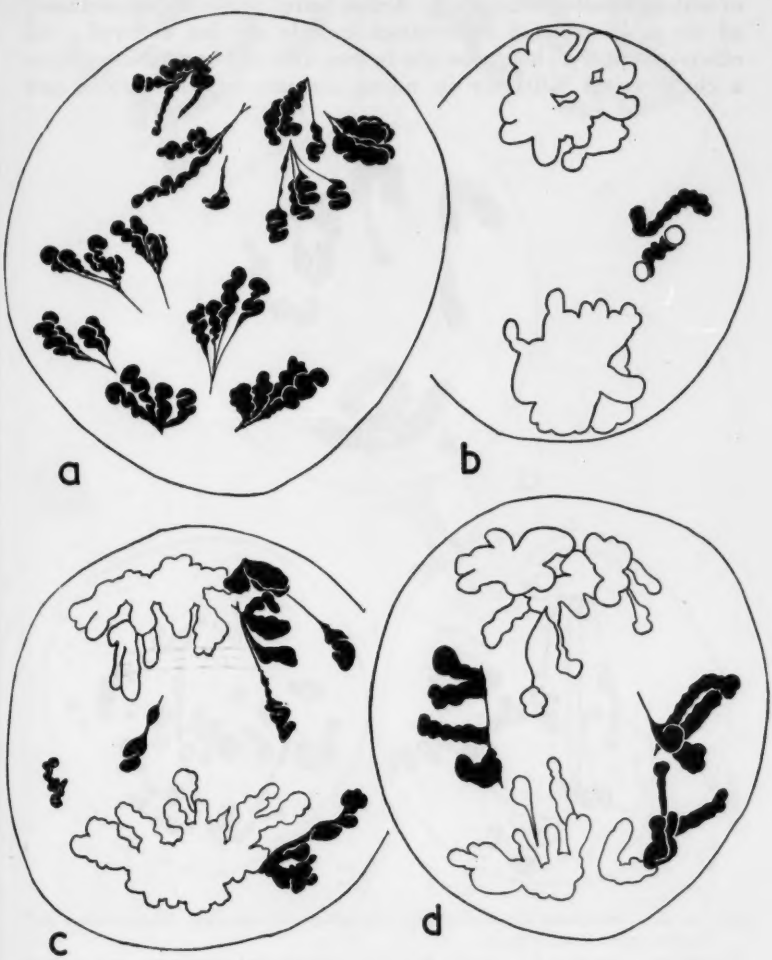

FIG. 10.—(*a*) Anaphase I : abnormal stretching and breaking at the centromere, irregular spiralisation. (1 : 100/3 min./24 hrs.)

(*b*) Univalent at telophase I : it has divided, and the chromatids show imperfect spiralisation. (1 : 100/3 min./24 hrs.)

(*c*) Anaphase I : breakages at the centromere regions. One acentric fragment shows nucleic acid starvation. (1 : 100/3 min./24 hrs.)

(*d*) Anaphase I : misdivision of the centromere, and stretching of the centromere region (1 : 100/3 min./24 hrs.)

The seriation of stages is not of course as exact with mustard gas as with terminable treatments. Action being, as we saw, cumulative, all the more deferred observations include the less deferred. All effects seen after 24 hours can also be seen after 48 hours. Nevertheless a characteristic difference in timing appears between mitosis and

FIG. 11 (*a*). Metaphase I: nucleic acid starvation and uncoiling, fragmentation and centric union (plate I, fig. 4).
(*b*) and (*c*). Anaphase I: acentric fragments, minutes and subminutes, bridges, and delayed separation of bivalents. (1 : 100/2 min./48 hrs.)
Note the contrast in amount of *visible* breakage at metaphase and at anaphase.

meiosis as shown by the method of correlation used in table 3. Only after 48 hours does the meiotic series begin to show the breakage which appears in the mitotic series after 24 hours. This difference is in keeping with the known prolongation of the prophase in meiosis as compared with mitosis.

Again the centromeres show no breakage or reunion at first metaphase of meiosis. Presumably they are not undergoing division

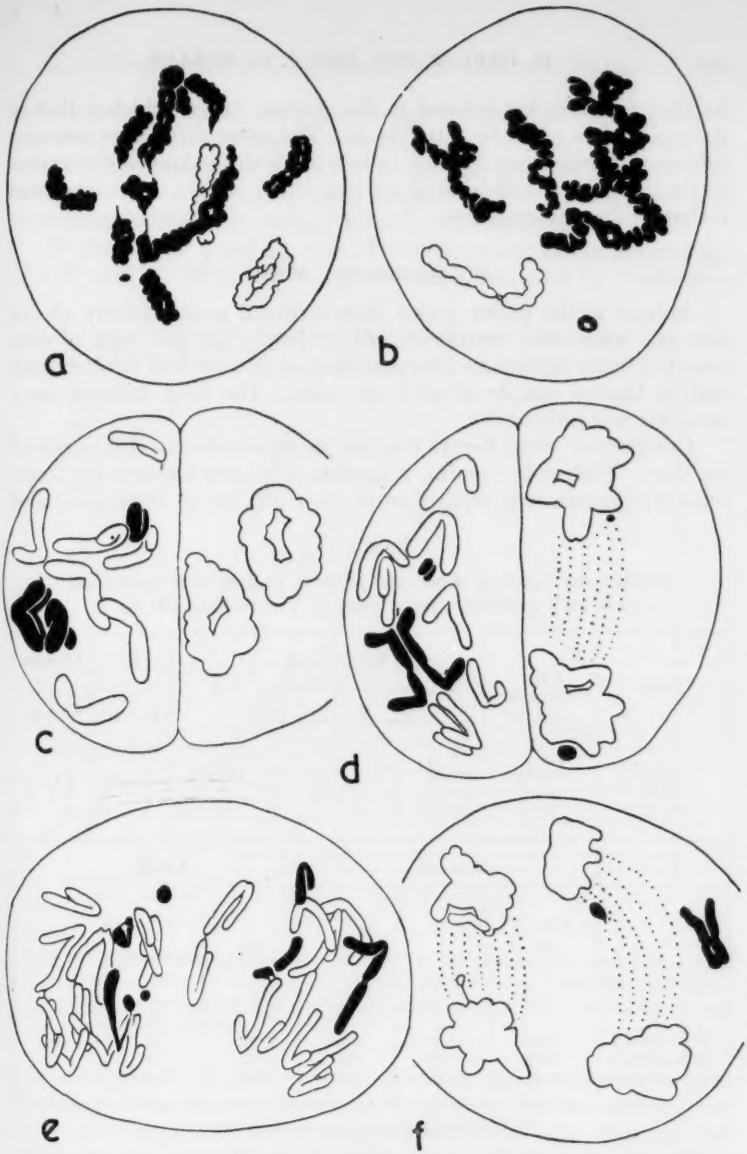

FIG. 12.—(*a*) and (*b*). Metaphase I: multivalents, unequal bivalents, and acentric fragments in cells with low breakage. (1 : 100/3 min./72 hrs.)

(*c*) Anaphase II: lack of synchronisation and of spindle formation on one side; the chromosomes have fallen apart but fail to move towards the poles. Dicentric chromosomes and acentric fragments are present. (1 : 100/3 min./72 hrs.)

(*d*) Abnormal distribution of chromosomes at anaphase II, with dicentrics. One member of the dyads is in telophase, while the other is in anaphase. (1 : 100/3 min./72 hrs.)

(*e*) Failure of wall-formation at the end of first division leads to the formation of a polyploid cell. Fragments are present. One chromosome shows stretching at the centromere, others show stickiness. (1 : 100/3 min./72 hrs.)

(*f*) Abnormal tetrad, without a wall between the cells. One chromosome is left behind with the centromere still undivided. (1 : 100/3 min./72 hrs.)

and are therefore less exposed to the greatest danger which is that of damaging themselves by misdivision. The other differences between mitosis and meiosis are likewise in keeping with the known differences in the timing of the division of the centromere and in the pairing and coiling of the chromosomes.

(ii) *Quantitative : Mitosis*

Mitoses in the pollen grains were recorded quantitatively 48, 72 and 120 hours after treatment. At 48 hours, 9·2 per cent. of cells were too badly injured for interpretation, so that we had to be content with a biassed sample of 90·8 per cent. The later samples were complete and unbiassed.

Comparison with X-rays enables us to standardise the mustard gas dose. With pollen grains, 2 minutes of 1 : 100 mustard gas treatment is approximately equivalent to 360 r (fig. 13, *cf.* Darlington and

TABLE 4

Breakage and reunion of chromosomes following treatment with mustard gas 1 : 100 (24°) 2 minutes ; pollen grains of T. bracteata (cf. *fig. 13*)

Time	Cells Total/broken	B″	R″		SR		B′	R′		Minutes	
			C_2	C_0	C_0	C_1		B′	B″	R″	R′
48 h.	110/85	137 (1)	2	4	57	26	163 (10)	73	24	3*	...
72 h.	40/32	50 (1)	3	1	24	14	37 (7)	12	2	8	2
120 h.	40/18	39 (2)	3	0	17	8	16 (7)	4	0	2	...

	B. p. cell			E.C.R.		
	B″	*m*	B′	R″	R′	
					B′	B″
48 h. contnd.	1·24	·09	1·48	·04	·44	·14
72 h. contnd.	1·25	·25	·92	·08	·32	·06
120 h. contnd.	0·97	·05	·40	·07	·25	...

* In addition 7 subminutes in one cell.

Notes.—B″ includes (i) free acentrics, (ii) those which have undergone R′ to give triradials, (iii) a small proportion of unscorable token breaks where unbroken ends have united by R′ to give triradials.

Brackets after B′ and B″ enclose additional centric breaks excluded from the analysis of ordinary breakage. E.C.R., Empirical Coefficient of Reunion of breaks.

La Cour, 1945). With *Drosophila* sperm Auerbach (unpub.) finds that 14 minutes of 1 : 10 mustard gas treatment, using the same chamber and dilution, is equivalent to about 5000 r. The measurement is by

chromosome breaks in the one case and by sex-linked lethals, which are mostly no doubt related to breakage, in the other. Allowing for a lower permeability of flies than flowers, the mustard gas treatment can, it seems, be measured more accurately than was expected and even quantitatively the analogy between the two treatments holds.

Breakage (table 4 and fig. 13). The average frequencies of breakage (B″, B′ and m) agree fairly well with those produced in *Tradescantia*

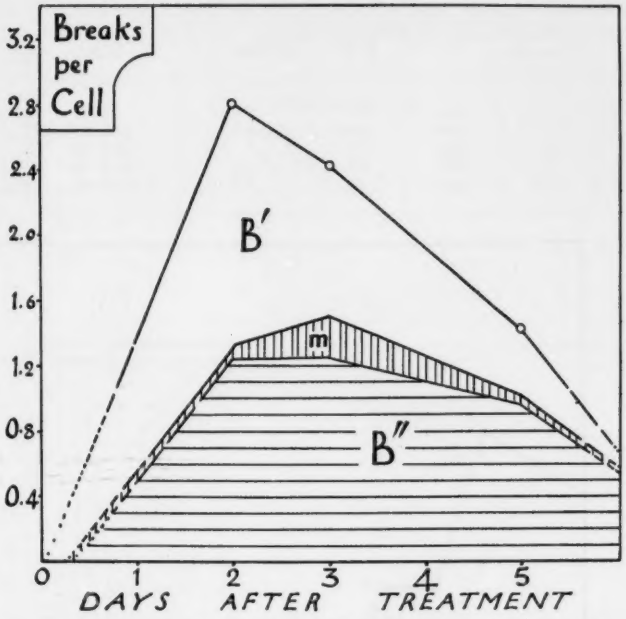

FIG. 13.—Graph showing breakage frequencies (B′, B″ and minutes) at different times after mustard gas treatment of pollen (with delayed effect) showing the analogy with X-ray treatment of roots (with differential development effect) as shown in Darlington and La Cour (1945), graph 6.

pollen by 360 r. The time curve, however, agrees rather with that produced at the same temperature by 45 r on *Trillium erectum* root-tips : the abrupt and clear changes found with X-ray treatment of pollen are no longer traceable. The reason for this is, again, that mustard gas, like low intensity radiation, has a continuous effect which acts like the dispersal due to different rates of cell development in the root-tip in blurring the differentiation by time. In addition to this external blurring, there is the internal blurring which is inherent in all breakage and comes about because the apparent chromatid breaks (B′) arising from treatment at all these stages are both original B′ and those surviving from B″ after R′.

The X-ray effects on pollen have shown that the true or direct chromatid breakage occurs at the most sensitive stage of all. The later mustard gas treatments are those having the highest effect at this stage. They are therefore consistent in yielding the highest breakage frequency as well as the highest proportion of chromatid breaks.

TABLE 5

Coefficients of reunion of B′ with one another and to give triradials B′ B″ R′ for all periods

B′ p. cell	B′	B′ R′	B′ B″ R′÷2	ECR′
1	52	...	3·5	+·07
2	76	40	4	·52+·05
3	33	17	1	·52+·03
4	28	12	1	·44+·04
5-10	47	20	3·5	·42+·07

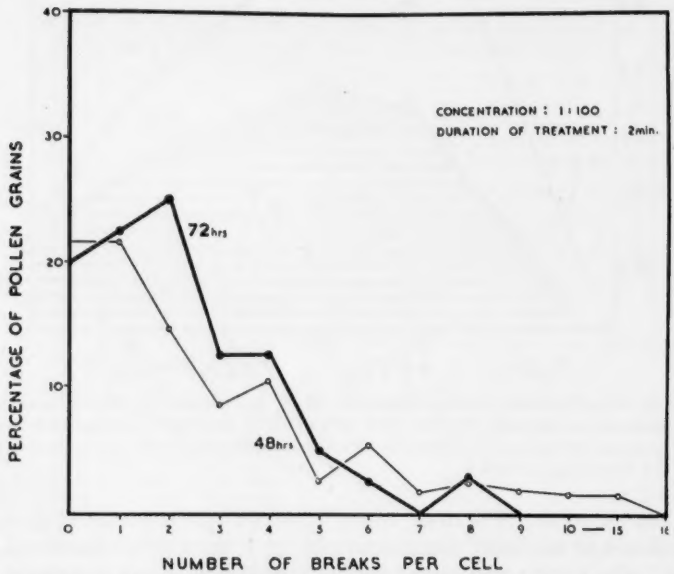

FIG. 14.—Frequencies of cells with different numbers of breaks (B′, B″ and minutes but not subminutes), 48 and 72 hours after treatment (not represented in a table). *Cf.* Darlington and La Cour (1945), graph 13.

Reunion (R″ and R′). The proportion of broken ends of chromosomes and chromatids which undergo reunion (the Empirical Coefficient of Reunion or ECR) is much lower than with high intensity X-raying. It corresponds with the values obtained at low intensities (table 4 and Koller, 1946).

The very low frequency of R″ as compared with R′ is due to the lower frequency of B″ than of B′, a frequency also which is spread over a longer time : to allow of reunion two breaks must be present in the same cell at the same time. This low R″ is another condition favouring the high proportion of triradials from B′-B″ unions already discussed. These have been separately recorded (table 5). These

TABLE 6

Frequencies of cells with different numbers of chromatid breaks and reunions (excluding B″ R′) for all three periods, showing retention of excess even breaks by excess of paired reunion. Reproduced in the graph, fig. 15. (Cf. *table 15B and graph 14 in Darlington and La Cour, 1945*)

	Numbers per cell										
	0	1	2	3	4	5	6	7	8, 9	10	T.
B′	95	32	38	11	7	2	1	3	...	1	190
R′	50	12	24	5	4	...	...	...	...	...	95
B′-R′	...	20	14	6	3	2	1	3	...	1	50

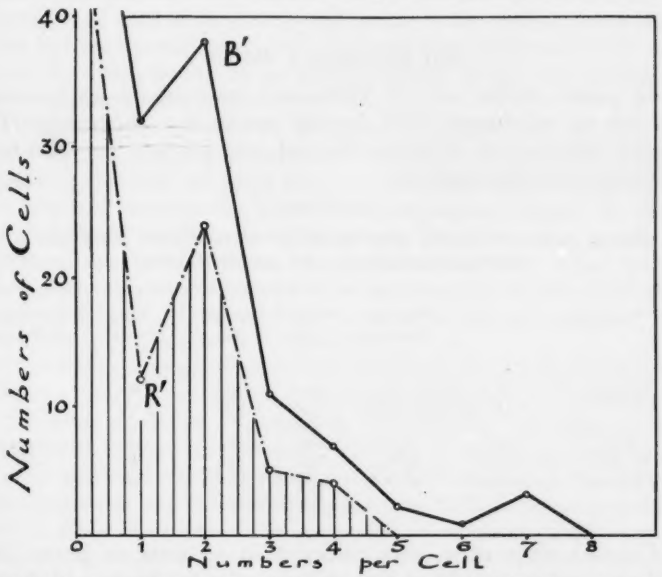

FIG. 15.—Frequencies of cells with different numbers of B′ and R′ (from table 6).

reunions are asymmetrical and therefore have a damping effect on the predominance of paired reunions and of the paired breaks which they preserve from restitution and consequent disappearance. The

zigzag ECR′ frequency curve with breaks per cell therefore disappears with mustard gas (*cf.* Darlington and La Cour, 1945, graph 16). Nevertheless the excess of even numbers, so remarkable in the X-ray results, is still detectable in the mustard gas experiments. The excess disappears, as it should do, when R′ is subtracted from B′ (table 6 and fig. 15).

Sister reunion (SR). The non-division of the end-gene is higher in the acentric than in the centric fragments produced by chromosome breakage (B″). For both classes it is highest in those cells having the lowest numbers of breaks (table 8).

Minutes (m). The relative frequencies of minutes at different stages following X-ray treatment at a favourable temperature can be calculated from the frequencies of large fragments and of their reunions on the assumption that the two breaks responsible for them are independent of one another (Darlington and La Cour, 1945, table 11). The same formula applies satisfactorily to the mustard gas data in giving expectations at different periods relative to one another (with a maximum at 72 hours). But the frequencies of minutes relative to the large fragments are four times as high as with intense X-ray treatment. This is perhaps another symptom of the concentration effect since it is also found with low-intensity X-ray treatment.

(*iii*) *Quantitative : Meiosis*

In pollen mother cells of *Tradescantia bracteata*, the six bivalents are ring or rod-shaped, each having one or two chiasmata. The chiasma frequency is between 1·60 and 1·75 per bivalent with very high terminalisation (table 7).

TABLE 7

Chiasma frequency in samples of 50 treated and untreated pollen mother cells of Tradescantia bracteata (*300 potential bivalents*)

Concentration	Exposure	Time after treatment	Total Xta	Terminal Xta	Xta per cell	Terminal coefficient
Control	P. C. Koller (1943)		480	464	1·60	*0·96*
	L. F. La Cour (1946)		526	520	1·75	*0·98*
Pure	1 min.	12 hrs.	475	471	1·56	*0·99*
1 : 100	5 mins.	24 hrs.	561	374	1·87	*0·66*
1 : 100	5 mins.	48 hrs.	582	231	1·94	*0·39*

Chiasma frequencies were recorded in 50 cells 12 hours after treatment with pure, and 24 and 48 hours after treatment with dilute, mustard gas. In the 12 hours' sample, the chiasma frequency was not significantly below normal. Evidently the chiasmata had been formed more than 12 hours before metaphase. Twenty-four hours and 48 hours after treatment with the weak mustard gas the number

of chiasmata is raised. Ring-shaped bivalents become commoner and terminal localisation is reduced. In other words, more chiasmata are formed and further from the ends. Since pairing is supposed to begin at the ends in *Tradescantia*, this result would follow if the time limit to pairing were merely postponed (Darlington, 1940). The reverse effect occurs in the procentrically localised pairing of *Fritillaria* at high temperatures : the time limit is advanced and localisation is increased (Barber, 1942).

An extension of the time available for the pairing of chromosomes would presumably arise from a delay in the accumulation of the proteins needed for their reproduction. It is therefore significant that Mather (1934) found an increased chiasma frequency, and La Cour (1946) widened distribution of localised chiasmata, following X-raying.

(6) CHEMICAL, RADIATION AND SPONTANEOUS EFFECTS

The distinction between spontaneous and temperature-induced errors on the one hand, and chemical and radiation errors on the other, seems to be in the more specific timing of the former kind. They include every type of error induced by mustard gas or slow X-rays save for misdivision and the formation of subminutes at mitosis ; and both of these may well be due to the concentration effect. Since in most spontaneous cases, however, as we saw, a specific error occurs by itself, owing to its being precisely timed, we should not expect concentration effects.

With breakage induced by X-rays or by mustard gas, on the other hand, we always have the whole series of nucleic acid and protein errors together. It is only by using low doses of either that some separation can be made between the primary and secondary effects. In short, the methods of breaking the chromosomes by chemical or irradiation treatment are, both of them, blunt instruments, have a less specific effect, and the chemical treatment a less precisely timed effect, than can be produced by the adjustment of abnormal environments and genotypes in " spontaneous " breakage.

The effects of mustard gas on the cell are revealed in the metaphases within 24 hours in two ways : (i) by a derangement of the nucleic acid metabolism, as shown by errors in the charging, reproduction and spiralisation of the chromosomes ; and (ii) by a derangement of the protein organisation, as shown by the centromeres and the spindle. The centromere appears as specially sensitive both in these experiments and in those of Auerbach as suggested by mosaics (1946) and by delayed errors at the following meiosis (1947). These errors are associated with other mutations probably due to loss of a chromosome segment. The whole syndrome could be due to misdivision at the first mitosis in the egg which would lead to loss of the short arm of the X. The breakage of the centromere, sometimes at prophase, sometimes at anaphase, provides the first experimental analogy with the

spontaneous breakages at the centromere previously seen in *Tradescantia* and *Tulipa*.

Since the centromere anomalies are the first to appear they are probably the last to occur in the cycle, that is just before metaphase. The occurrence of errors in its development and its actual breakage under the influence of both radiation and chemical treatment at prophase, the very stage when the rest of the chromosome is immune to breakages, is evidence of its structure and development. At this stage the rest of the chromosome is taking up a charge of nucleic acid which is giving it a rigid spiral framework. The centromere, on the other hand, is losing what charge it had as it prepares to organise by direct action the protein arrangement of the spindle. The chromosome is taking up protective covering as its genes give up their activity. The centromere is losing its protective covering as its genes take up their activity. Hence their reciprocal change of sensitivity equally to chemical and to radiation attack (Darlington, 1947).

The centromere effect may well be characteristic of all radiation treatment of prophase, but where intense doses have to be delivered immediately before metaphase in the sensitive stage, centromere damage is bound to be smothered by the nucleic acid effect. Prolonged treatment with low intensity X-rays or with mustard gas avoids this confusion.

After 24 hours the effects of action on nucleic acid and on the centromere disappear and the specific effect on chromosome breakage is left. This transition is parallel with that between the primary and secondary effects of X-ray treatment. With low X-ray doses, the transition can be more easily followed than in the present mustard gas treatments, but the principles are evidently the same.

The breakage effects of mustard gas and low intensity X-ray treatment are parallel. They both differ from high intensity dosage owing to the cumulative action of the slow treatments whether with the radiation or the poison. This action makes itself felt in two ways :—

(i) There is a low-intensity effect shown by low reunion. This does not, however, account for the low frequency of interchanges found by Auerbach, for in any case there is no reunion at all in *Drosophila* sperm.

(ii) There is a concentration effect shown by the formation of subminutes as well as by the dispersed frequency distribution in cells (fig. 14). The later part of each treatment is acting on chromosomes in a substrate already altered by the earlier part. Sax has inferred (1941, 1942) that acentric fragments are less breakable than centrics under a second X-raying. It seems rather that the reverse is true, especially in view of the characteristically higher sister reunion of acentrics ; but in any case the effects of a prolonged treatment are likely to

depart from the randomness of a purely physical experiment indicated by intense treatments, or treatments of simpler systems such as viruses or *Drosophila* sperm.

Even more may be learnt about the conditions of breakage from the comparison of the frequencies of reunion. The mustard gas effects agree with the X-ray effects, and contradict the spontaneous effects with *Tulipa fragrans*, in regard to sister-reunion. They do so in two ways (table 8, fig. 16) :—

(i) They show a *higher* sister-reunion in acentrics. This is obviously because the loss of the centromere upsets the nucleic acid charging and leads to non-division of the broken end-gene. The spontaneous breakage goes clean contrary. There is *lower* sister-reunion in acentrics. The reversal means that these breaks themselves are derived from nucleic acid defects due in turn to errors of the centromere : its loss is therefore more of a help than a hindrance.

(ii) They show *less* end-gene defect in cells with more breaks. Noticing this correlation in our mustard gas data, we re-examined the X-ray data and found they agreed. The explanation (which is not important for our present purposes) is probably that the disadvantages of being broken, and also of having no centromere, are now shared amongst numerous ends. With spontaneous breakage, on the contrary, there is *more* end-gene defect in cells with more breaks shared amongst numerous ends. Again the spontaneous breakage goes clean contrary to the induced and for the same general reason : the greater damage is a symptom of greater error in the cell, error in the nucleic acid metabolism.

The centromere and nucleic acid errors which are induced by the X-rays and mustard gas are induced with great rapidity and are invariable concomitants of the breakage although they may no longer be in evidence at the time when resting stage breaks reach metaphase. It is therefore of great importance to know that, while spontaneous breakage is dependent on these errors, induced breakage, of both types is independent of them. In other words, it is direct rather than indirect.

We now have to examine an assumption at the opposite extreme. It has been held that the radiation breakage of chromosomes arises from the direct, or almost direct, action of the ionising particles on the gene-string. Such a view could hardly be maintained for a chemical mutafacient. The close relationship between X-ray and mustard gas effects, therefore, makes it worth while to re-examine the direct ionisation theory.

TABLE 8

Sister reunion related to breakage frequency in individual cells (cf. *fig. 16*)

(*a*) Following mustard gas treatment in the experiments recorded in table 4

B′ p. cell	B″	B″ R″	SRC_0	EC : SRC_0	SRC_1	EC : SRC_1
1	46	46	26	·57	13	·28
2	50	47	22	·47	9	·19
3	45	40	19	·47	10	·25
4-5	54	53	22	·41	11	·21
6-10	31	28	9	·32	5	·18
Total . .	226	214	98	(·40)	48	(·23)

(*b*) Following spontaneous breakage in two classes of cells with low and high breakage in *Tulipa fragrans* (Darlington and Upcott, 1941)

Cells	B″	B″ p. C.	R″	B″-R″	SRC_0	EC : SRC_0	SRC_1	EC : SRC_1
11	49	4·5	21	28	3	·11	4	·14
4	44	11·0	14	30	8	·27	14	·47

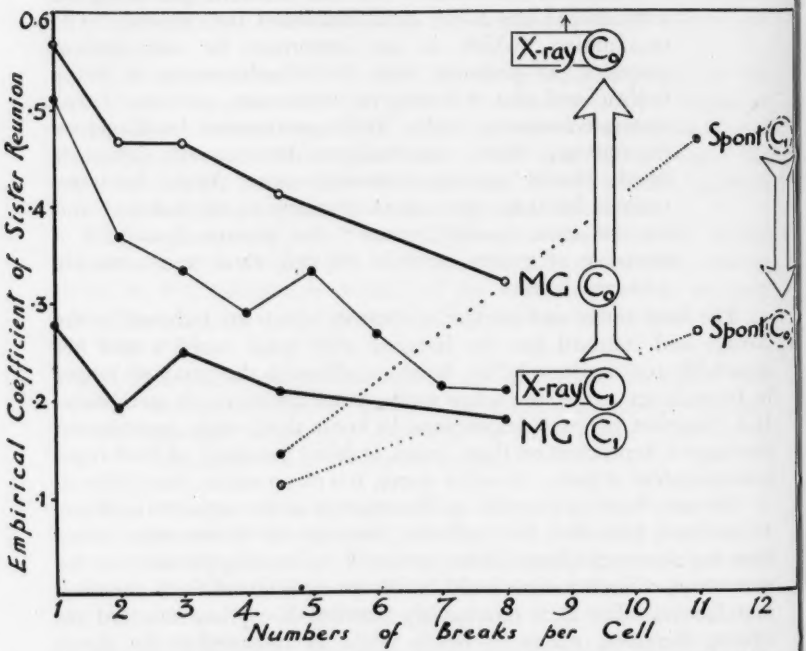

FIG. 16.—Graph showing the relationships of sister-reunion in pollen grains with high and low breakage in centric and in acentric fragments following :—

(i) Mustard gas treatment of *Tradescantia bracteata*. (Table 8 (*a*).)

(ii) X-ray treatment of *Trillium erectum* (calculated from Darlington and La Cour (1945), table 16, p. 242.)

(iii) Spontaneous breakage in *Tulipa fragrans*. (Table 8 (*b*).)

(7) THE TARGET THEORY

The view that a single ionisation within a given target will directly break a chromosome thread has been pursued farthest by Lea (1946). His argument rests on experiments of Sax, Catcheside, and other workers who have followed Sax's experimental methods and accepted his conclusions as a basis of further analysis. These experimental methods suffer from certain shortcomings of design and control. They depend on the assumption that not merely genes but also cells and organisms may be relied upon to react to treatment according to purely physical laws, to behave as targets when hit, and in short never to introduce any new variables into an experiment unless invited to do so.

This point of view was very advantageous when Jacques Loeb first revealed its possibilities, and it is still the foundation of a large part of what passes for experimental biology. But to-day it can lead the unwary physicist (or bio-physicist) into pitfalls. For example, *temperature* is of no consequence if the effect of ionisation is direct. For this reason, presumably, temperature control has been largely dispensed with in the experiments used by Lea as evidence. Only *time* has been controlled. But time means nothing for living organisms when the temperature is unknown.

By contrast with these fundamental fallacies of method, most of which have already been examined (Darlington and La Cour, 1945 ; Koller, 1946), the fallacies of inference and classification derived by Lea from Sax are superficial. They are, however, destructive of the analysis that rests on them. In the first place Sax (1939) showed, and the present experiments with the prolonged action of the poison entirely agree, that a spreading of irradiation with a low intensity over a long period resulted in fewer new unions, that is fewer rearrangements of chromosomes. Sax concluded that breaks " healed " quickly so that, unless two were formed in a cell at once, they could not reunite in a new way. Later Darlington and La Cour (1945) showed that healing *did* not occur in the resting nucleus, Muller (1940) and McClintock (1942) having already shown that it *need* not occur at all for many cell generations. Now, of course, we can go much further. The present experiments show, not only that breaks do not heal, but also that *un*broken ends may *un*heal. Darlington and Upcott (1941) had also explained the reduction of reunion with prolonged treatments by assuming that new reunions had to compete with old reunions : restitution would forestall rearrangement unless more than one break happened at once. This assumption is vindicated by the increase of delayed reunion and the formation of triradials with the prolonged mustard gas treatment.

The assumption of competition, thus strengthened, enables us to rectify another early error. Already, in 1938, Sax, attempting to apply Mather's principle that B″ are breaks of chromosomes before the

split and B′ of chromatids after the split, had to explain why the same chromosome could show both B″ and B′. He assumed that, in such cases, the B″ were spurious and the result of two chromatids being broken by one "hit" (on the direct ionisation hypothesis). These "isochromatid breaks" have done service for many years and figure largely in Lea's work. But, unfortunately, no one who has recorded them has ventured to define where their spuriousness begins. As we see, in the present work, B's can, and do, unite with B″s to give triradials. Hence, as pointed out by Darlington and Upcott (1941), any B′ can be the result of the reuniting of one chromatid of a B″ which leaves its sister unmended. And, further, it is the B's that are spurious and not the B″s (*cf.* fig. 17).

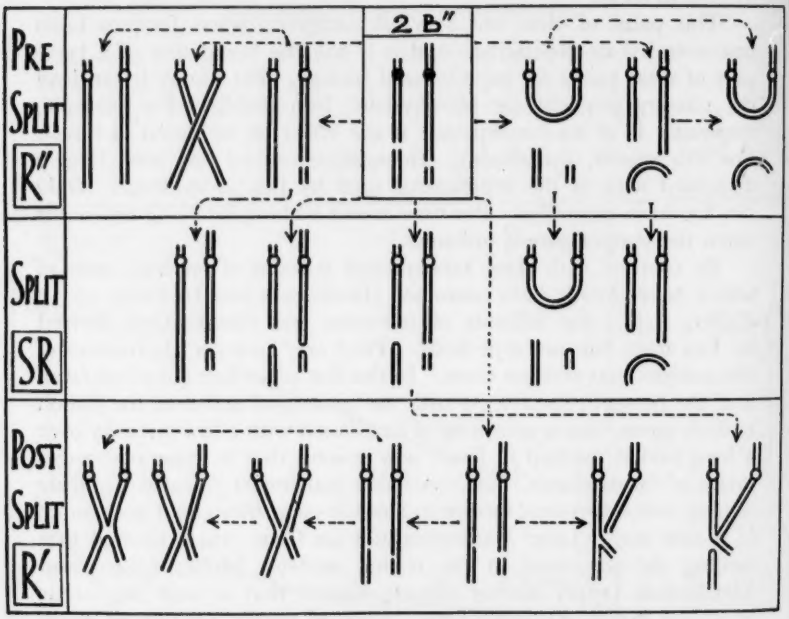

FIG. 17.—Some of the possible successions of changes that may follow two breaks (B″) in two chromosomes owing to restitution, R″, SR and R′. The pre-split stage is represented as split for ease of comparison. Each stage may be final and hence observable at the ensuing metaphase.

Note that SR is, so far as we know, independent on the two sides (centric and acentric) of a break.

What Sax's low intensity experiment proved, therefore, was not that broken chromosomes *healed* on an average within so many minutes, but that treated chromosomes *broke* on an average within so many minutes. This discovery, although it has hitherto been assumed by Lea, as well as by Sax, owing to the supposed indisput-

ability of the direct ionisation hypothesis, is in fact most important and we can now build on it.

We now have four established positions. (1) The chemical and the radiation-induced breakage show the closest similarity and the reunions following them likewise. (2) Both of them happen only to chromosomes, or to parts of chromosomes, that have been stripped of nucleic acid, *i.e.* subject to the chemical surroundings of the genes at the time. (3) Both of them occur at the same time as injury to the nucleic acid metabolism, nevertheless both of them are independent of it. (4) The X-ray breakage happens within a few minutes of impact, the chemical breakage therefore presumably likewise. On these bases it is scarcely possible to doubt that both X-rays, as suggested by Muller (1940), and chemical breakage are immediately determined by chemical processes which in the X-ray case intervene between an ionisation and breakage. In the study of these chemical processes the work of Dale (1942), Mitchell (1943), Hevesy (1946), Latarjet (1946) and McAulay and Ford (1947) has been important and the mustard gas experiments may provide yet another means of attack.

(8) SUMMARY

1. Mustard gas applied to anthers as a dilute vapour for 2 minutes has a prolonged toxic action on the cell, and especially on the nucleus, like that of continuous low intensity radiation.

2. Its effects on mitosis and meiosis may be set down to injuries to both the nucleic acid metabolism and the protein structure. The first symptom is in a weakening of the centromere which later may break in the prophase or misdivide at anaphase. The second is in the stickiness of the nucleic acid charge on the chromosomes which affects their spiralisation and their reproduction. The third is in the breakage of the gene-string during the resting stage.

3. The breakage of the centromere at prophase of mitosis is due to its loss of nucleic acid protection at this stage when the rest of the chromosome is becoming protected. At the prophase of meiosis, however, the chromosomes are broken both before and (with different results) after pairing. These effects are all reproduced by X-raying but centromere breakage requires low intensities to be made visible.

4. Resting-stage breakage also corresponds with low intensity X-raying (i) in the low frequency of chromosome reunions (R″), (ii) in a concentration effect whereby minutes are broken to give subminutes and (iii) in a high frequency of triradials formed by the delayed union of chromosome breaks (and even of unbroken ends) with chromatid breaks.

5. These observations confirm the previous conclusions (i) that breaks do not heal in the resting nucleus (ii) that the intensity effect is due to competition for reunion and proves only that breakage is immediate, (iii) that apparent chromatid breaks can be derived from

chromosome breaks by delayed reunion and (iv) that "isochromatid breaks" are fictitious.

6. Mustard gas and X-ray breaks differ from spontaneous breaks in the higher frequency of sister-reunion amongst acentrics. They also differ in having a lower frequency of sister-reunion in cells with more breaks. Both types of induced breakage are therefore independent of the simultaneous injury to the nucleic acid system on which spontaneous breakage is dependent.

7. The close similarity of chemical and physical breakage, and of their results, favours an indirect rather than a direct action of the ionising radiations on cells with a differentiation of nucleus and cytoplasm. Both these types of induced breakage are more complex in their conditions than spontaneous breakage.

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Plate I and II.—Photomicrographs of Tradescantia bracteata treated (—72 hours) after with mustard gas. × 2200.

Plate I.

Fig. 1. Pollen mother cells.

Figs. 1 and 2.—Laggards. (1 : 10/2 minute/72 hrs.)

Fig. 3.—Mitotic division of chromosome in a univalent with irregular nucleic acid starvation. (1 : 10/2 min./72 hrs.)

Fig. 4.—Fragmentation of chromosome and starvation at metaphase. (1 : 10/2 min./48 hrs.). CX. Text fig. 11a.

Fig. 5.—Irregular spindle; lack of synchronisation of two second division anaphases. (1 : 10/2 min./72 hrs.)

Fig. 6.—Accessory fragments forming a secondary plate where the cell wall should be laid down. (1 : 10/2 minutes/72 hrs.)

Plates I and II.—Microphotographs of *Tradescantia bracteata* treated 12-72 hours earlier with mustard gas. ×2200.

Plate I. Pollen mother cells

FIGS. 1 and 2.—Laggards. (1 : 10/2 min./12 hrs.)

FIG. 3.—Misdivision of centromere in a univalent with irregular nucleic acid starvation. (1 : 100/2 min./12 hrs.)

FIG. 4.—Fragmentation, non-spiralisation and starvation at metaphase. (1 : 100/2 min./48 hrs.). *Cf.* Text fig. 11*a*.

FIG. 5.—Irregular spindle; lack of synchronisation of two second division anaphases. (1 : 100/2 min./72 hrs.)

FIG. 6.—Acentric fragments forming a secondary plate where the cell wall should be laid down. (1 : 100/2 min./72 hrs.)

Plate II. Pollen grain mitosis

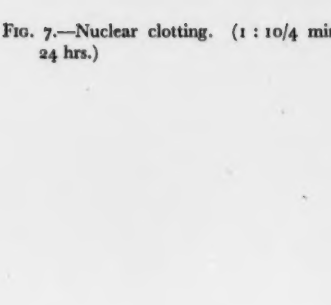


FIG. 7.—Nuclear clotting. (1 : 10/4 min./24 hrs.)

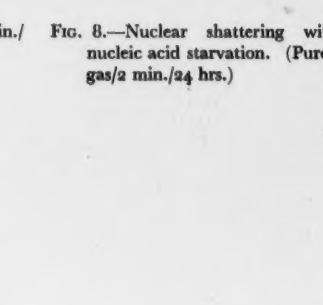


FIG. 8.—Nuclear shattering with some nucleic acid starvation. (Pure mustard gas/2 min./24 hrs.)

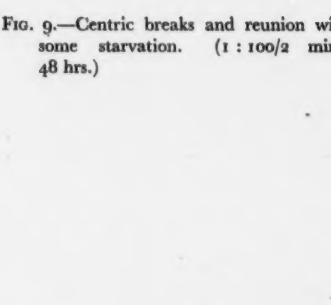


FIG. 9.—Centric breaks and reunion with some starvation. (1 : 100/2 min./48 hrs.)

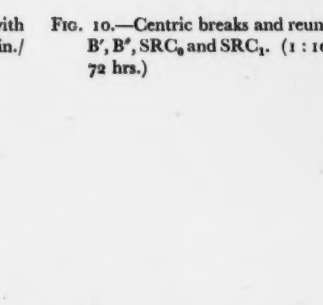


FIG. 10.—Centric breaks and reunions, with B′, B″, SRC_0 and SRC_1. (1 : 100/2 min./72 hrs.)

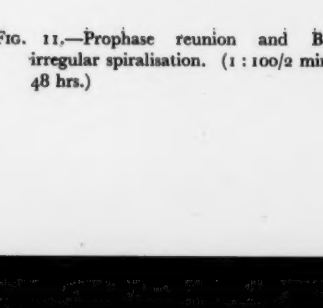


FIG. 11.—Prophase reunion and B″; irregular spiralisation. (1 : 100/2 min./48 hrs.)

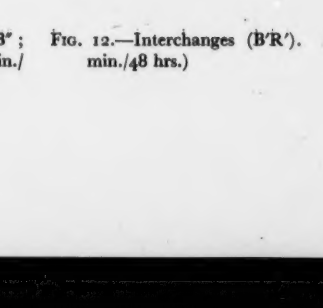
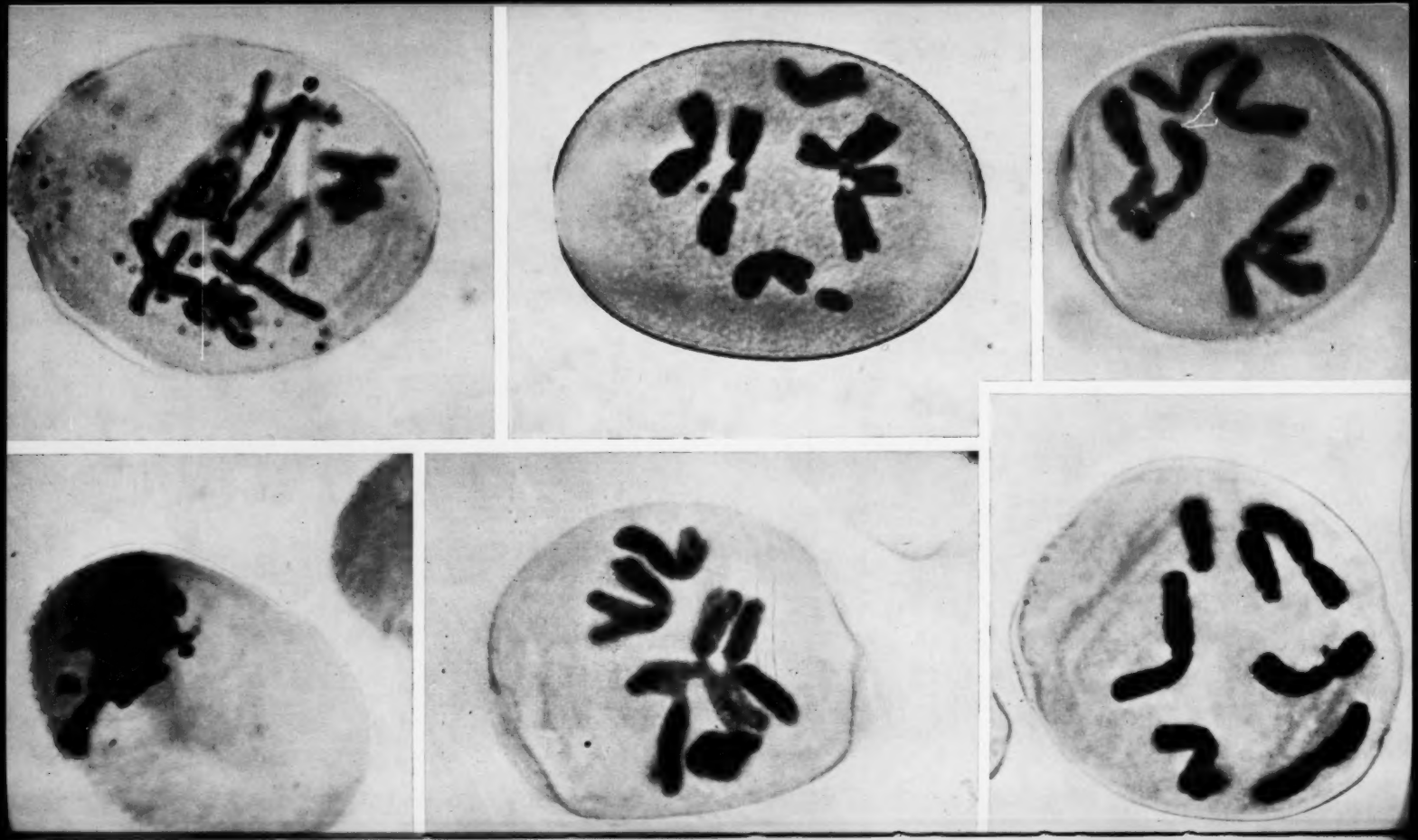


FIG. 12.—Interchanges (B′R′). (1 : 100/2 min./48 hrs.)



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THE EFFECTS OF SELECTION FOR YIELD IN WHEAT

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Received 3.ix.46

INTRODUCTION

In the introductory paper to this series, Frankel (1946) has discussed the general problems encountered in selection for total yield. Once the maximum resistance is reached to factors which limit yield and whose effects are observable, further progress can be achieved only by increasing the components of yield.

The principal difficulty at this stage arises from the fact that yield components are so subject to environmental variation that the recognition of favourable genotypes is difficult even in replicated yield trials. Genetic concepts require segregating populations greatly exceeding in number those which can normally be included in such trials. Hence the efficiency of selection of single plants and their immediate progenies constitutes a major problem in selecting for yield itself.

This paper examines the efficiency of selection of single plants and their progenies in a practical plant breeding scheme on wheat. It seeks an answer to the following questions :—

A (1) Are the effects of selection in one generation, on the yield of the next, large enough to overcome the effects of environmental variation ?

(2) Is selection for components of yield likely to make greater advances than selection for yield alone ?

(3) Is selection for an agronomic character, such as straw length, itself not a component of yield, likely to have an effect on yield in the following generation ?

B (1) Is selection by eye-judgment, instead of actual measurement, effective in retaining a large proportion of the material which would have been selected by measurement ?

MATERIAL AND METHODS

The material used was part of an F_2 generation of a compound cross (F_1 Holdfast × Tainui) × Cross 7.

The use of crosses between two F_1s—or in this case an F_1 and a third variety—enables the desirable characters of several varieties to be brought together for simultaneous selection. For gene differences

in the first crosses, a compound F_2 is in fact an F_3; for those in the second cross it is an F_2. Hence a compound F_2 possesses high intra-plot segregation combined with inter-plot segregation, the measure of both depending on the genetic differences between the parents concerned.

In practice, more often than not selection in self-fertilised plants does not commence until an advanced hybrid generation has been reached. Since in this study mean values per plot are used in the F_3 generation, the effects of selection in F_2 and, say, F_8 would differ only if F_3 plots possessed genetic skewness due to dominance.

Parents

Name	Origin	Agronomic characteristics	Yield and yield components 1944			
			eng	e	n	g
Holdfast	Produced by Plant Breeding Institute, Cambridge	Yield—usually below or close to—Cross 7. Shattering of grain a serious fault	...	3·08	...	36·7
Tainui	Produced by New Zealand Wheat Research Institute. Selection from Portuguese sample	Yield—close to or above Cross 7. Used as a spring wheat	3·43	3·77	17·2	53·8
Cross 7	Produced by New Zealand Wheat Research Institute. Cross: Tuscan × White Fife	Principal standard wheat in New Zealand	4·22	4·51	23·7	40·0

Cf. p. 226

The tendency to shattering, inherited from Holdfast, materially weakened the F_2-F_3 correlations.

History of cross

Year	Generation	Segregation
1940	Cross: Holdfast × Tainui	None
1941	Compound cross: (F_1: Holdfast × Tainui) × Cross 7	None
1942	Compound F_1	Intra-plot or inter-plant
1943	,, F_2	Intra-plot and inter-plot
1944	,, F_3	Intra-plot, intra-family, inter-family

A block of five compound F_2 plots and their F_3 progeny was studied. In F_3, 200 progenies of both unselected and selected F_2 plants were raised, affording a test for inter-plot selection in F_3 and for selection in F_2. In the test for eye-judgment, the F_2 was selected by two, the F_3 by three observers, working independently.

Throughout the discussion the following terms and symbols are used :—

Plot : Progeny of a single plant—contains about 50 plants.
Family : Group of F_3 plots containing progenies of plants from a common parent plot.
e : number of ears per plant.
n : number of grains per ear.
g : average weight of one grain in mg.
ng : weight of grain per ear in mg.
eng : yield per plant (F_2) or mean yield per plant (F_3).

A. THE EFFECT OF SELECTION IN F_2 ON YIELD IN F_3

The F_2 plants were arrayed plotwise and again " over all plots," in order of their measurements of *eng* and its components. Selection limits were set at 10 and 20 per cent. of each array. The mean yields per plant of the F_3 progenies raised from these top fractions were averaged, and related to the mean yield per plant of the whole corresponding F_2 family (table 1).

TABLE 1

Effect of selecting for eng *and its components in* F_2 *on* eng *in* F_3

F_2 plots or F_3 families	Number of F_2 plants and F_3 plots	Level of selection	Percentage increase over unselected F_3 family means by selection for *eng* and its components in F_2					Per cent. increase by selecting in F_2 for short straw
			eng	e	n	g	ng	
		Per cent.						
1659	42	10	16·5	5·8	12·0	−11·0	17·1	−10·5
		20	13·1	8·8	12·0	−15·3	10·1	0·2
1660	45	10	−2·7	−12·1	−13·0	−13·0	−15·7	−8·0
		20	−7·7	−9·5	−8·0	−13·9	−8·0	−5·4
1661	34	10	34·5	26·8	5·6	−12·7	4·4	−16·3
		20	13·8	6·4	11·7	−5·1	19·4	3·8
1663	35	10	34·5	2·0	12·2	11·4	18·1	−11·9
		20	−1·9	...	5·5	19·6	14·0	−14·1
1664	44	10	−2·5	−3·2	−5·9	1·4	−4·2	10·4
		20	−3·1	2·6	−3·6	−5·4	3·6	9·0
Over all plots	200	5	9·4	11·7	−5·2	14·6	−11·5	−22·0
		10	13·1	8·5	−4·4	−14·6	3·1	−11·8
		20	10·8	2·5	−6·7	...	6·8	−5·8

(1) *Efficiency of selection for yield* (eng)

(*a*) *Selection within plots* (plantwise). Selection for *eng* in F_2 was successful in raising the mean yield per plant in only two of the five

F_3 families. Owing to the small number of F_2 plots tested this result is inconclusive (table 1, column 4).

(*b*) *Selection between plots* (plotwise). The correlation between the mean yields per plant of the F_2 plots and the resulting F_3 families ($r = \cdot 79 \pm \cdot 17$) indicates that selection between plots was justified.

(*c*) *Selection over all plots* (plantwise). The selection in F_2 over all plots was successful in raising the mean yield of the progenies of selected plants above the mean yield of the unselected F_3 population (table 1, column 4, bottom line). This was achieved, in part, by the differential selection between plots, since those with the highest means had greater than random representation in the over-all sample. This fact, however, fails to account for the whole of the increase of 10·8 per cent. at the 20 per cent. selection level; for a random sample taken within plots in the same proportions raises the mean of the F_3 by only 3·4 per cent. This indicates some success of plantwise selection.

(2) *Efficiency of selection for yield components* (e, n, g)

Using Fisher's (1936) concept of discriminant functions, H. F. Smith (1936) suggested a method for selection based on the inequality of environmental variances of the individual components of yield. He found that between wheat varieties, selection based almost entirely on grain weight was most effective. Whether this principle could be used to discriminate between individuals of an early hybrid generation has yet to be established.

In this material (table 1, columns 5-8) the only yield component which consistently varies with yield is *n*. The range of variation of *n*, however, is in general below that of *eng*. Since *n*, which requires a count, is a good deal more cumbersome to establish than *eng* which is based on weight, no advantage would accrue from the use of *n* in preference to that of *eng*.

(3) *The effect of selection in F_2 for a character, not itself a component of yield, on yield in F_3*

Table 1, column 9, shows that straw length and yield are not wholly unconnected; yet the direction of the correlation, as well as its strength, varies from plot to plot. The effect on yield of selection for short straw is therefore unpredictable. In this material the conclusion is justified that should straw length be of relevance, selection for yield is not likely to be affected seriously by a consideration of this character in addition to yield.

B. THE EFFICIENCY OF SELECTION BY EYE-JUDGMENT

From the foregoing sections it is evident that in this material *eng* is the only reliable measure in selecting for yield. However, the weighing of each plant is cumbersome and limits the size of the

material which can normally be handled. Selection by eye-judgment on the other hand is comparatively rapid and facilitates selection from a large population. Its efficiency in selecting for yield is examined in this section.

(1) *Efficiency of selection by eye in* F_2 (plantwise)

(*a*) *Selection within plots.* Fig. 1*a* shows that both workers tended to select plants with yields well above the mean yield of each plot.

(*b*) *Selection between plots.* The proportion of plants selected within each plot varied with the mean yield per plot (fig. 1*a*). This difference in intensity of selection, between plots, resulted in an over-all increase in mean yield per plant regardless of the efficiency of single plant selection (*cf.* p. 226 (*c*)).

(*c*) *Over all plots.* The relative efficiency of selection was measured by the proportions of the highest yielding twentieth, tenth and fifth (by measurement) of the whole population, which were detected by the selectors; that of rejection, by the proportions rejected in the remainders. There was no attempt made to group the selected plants by eye-judgment (table 2). The distributions of the unselected population, and the plants selected by either A or B are shown in fig. 2.

TABLE 2

Efficiency of eye-selection and rejection in F_2

Level of top fraction	5 per cent.		10 per cent.		20 per cent.		100 per cent.
	Selected	Rejected	Selected	Rejected	Selected	Rejected	Selected
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Selector A	67	86	61	88	45	90	17
„ B	92	90	78	93	47	94	14
A+B	67	93	57	95	34	96	10
A or B	92	82	83	85	58	88	21

$$\text{Selected} = \left(\frac{\text{selection by eye}}{\text{selection by weight}} \times 100\right) \qquad \text{Rejected} = \left(\frac{\text{rejection by eye}}{\text{rejection by weight}} \times 100\right)$$

The success of selection at the 5 per cent. level and the partial failure at 10 and 20 per cent. is due to the wide difference between the yields of a few top plants and the mean yield of the population, as illustrated in fig. 1. With a decreasing intensity of selection, this difference also decreases, and selection becomes more difficult. At the 20 per cent. level, the yields of the majority of the plants differ only slightly from the population mean and selection is more or less random. At this stage personal bias would seriously modify selection.

Selector A, while retaining a larger proportion of the whole population, was less efficient than B in detecting the top fraction,

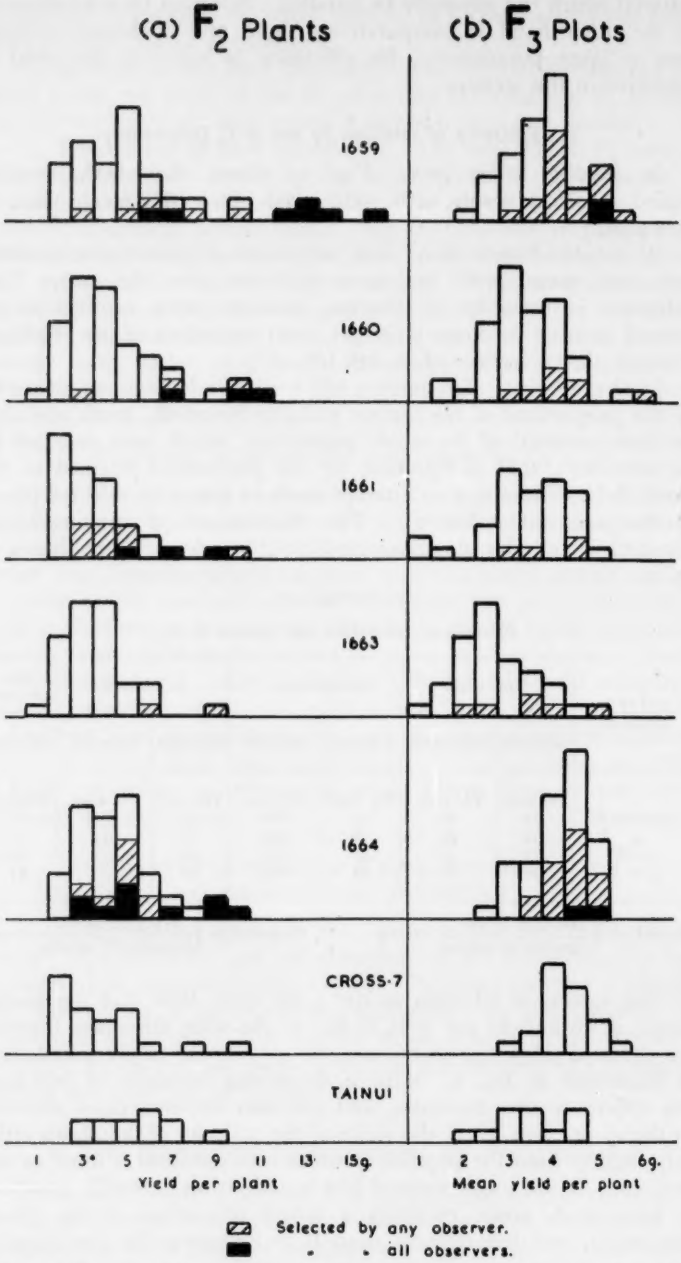

FIG. 1.—Frequency histograms showing distribution of selections by eye. Plantwise in F_2 and plotwise in F_3.

and in rejecting unwanted material. This may have been due to a bias in selecting for plant type. A comparison of the means of yield components and mean straw length of selected and rejected plants

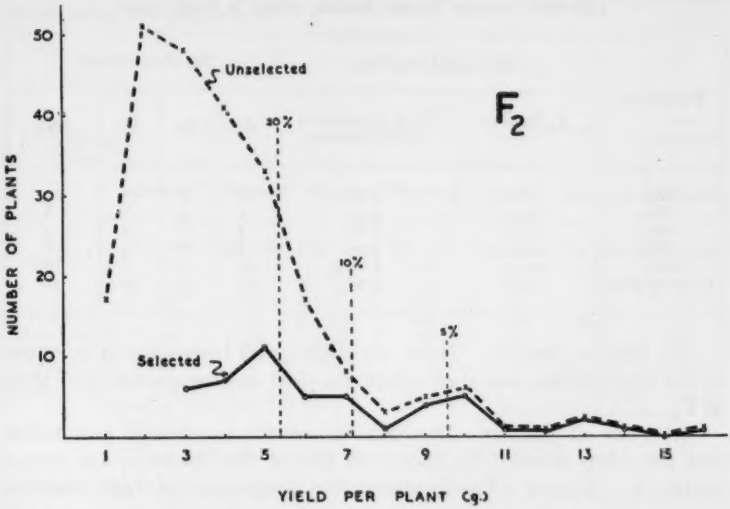

FIG. 2.—Distributions of the unselected F_2 population and the plants selected by eye.

shows that selector A tended to give greater weight to short straw than selector B. In this generation short straw was negatively correlated with yield, which may explain in part the lower efficiency of A. Both selectors rejected a few high yielding plants with signs of shattering.

(2) *Efficiency of selection by eye in F_3* (plotwise)

Each F_3 family contained five standard plots of Cross 7, two of Tainui and two of Holdfast. It was assumed that variations between the yields of consecutive Cross 7 plots might indicate fertility trends and provide the observer with a standard of comparison. It was found that the mean yield per plant of the F_3 families was correlated with the mean yield of the corresponding Cross 7 standard plots ($r = \cdot 85$). Adjustment of the F_3 mean yields according to the variation between the sets of standards removes most of the variation between families.

The number of plots selected from each family show that no observer used the Cross 7 plots as an aid to selection between families (table 3).

(*a*) *Within families.* Environmental variations within plots and between plots render small differences between plots difficult to detect; especially when they are not adjacent. Yet, as in F_2, all

observers tended to select plots with yields above the mean yield of each family (fig. 1*b*).

TABLE 3

Differential selection between families, related to family mean

Family no.	Mean yield per plant		No plots selected			
	F_3 families	Cross 7 standards within families	A	B	C	ANY
1659	3·74	4·39	13	8	13	20
1660	3·39	4·09	1	3	4	8
1661	3·39	3·84	3	1	2	4
1663	2·70	3·94	4	1	3	6
1664	4·11	4·80	14	4	8	19
Over all plots	3·50	4·22	35	17	30	57

(*b*) *Between families.* There are differential intensities of selection in the five families, but their effects on yield are less pronounced than in F_2.

(*c*) *Over all families.* Distributions of the unselected population and the plots selected by each and any of the observers are shown in fig. 3. Selection has increased the proportion of high yielding

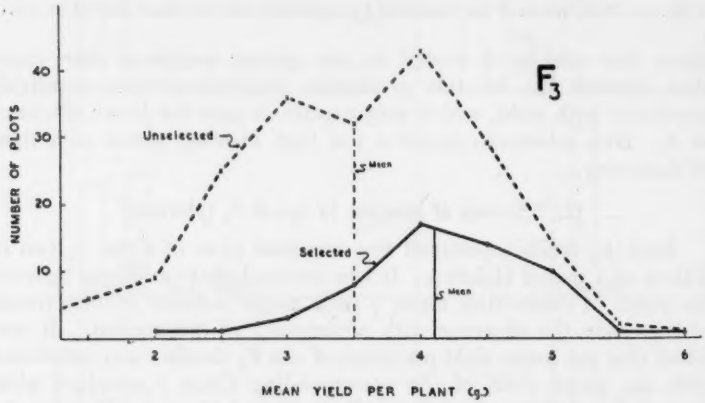

FIG. 3.—Distributions of the unselected F_3 population and the plots selected by eye by each and any of the three observers.

plots. However, here selection covers the full range, for estimating plot yields is more complex than estimating plant yields.

The primary aim of selection at this stage is to retain as much as possible of the top fraction ; a secondary aim is to reject as much as possible of the remainder. Table 4 shows how the judgment of the three selectors was influenced by the two considerations. A

and C retained, roughly, twice as many plots as B. The latter selector, however, secured only half the proportion of high yielding plots, whilst his efficiency of rejection was not much better than that of A and C. This argues for a relatively low intensity of selection when using eye-judgment.

TABLE 4

Efficiency of eye-selection and rejection in F_3

Level of top fraction	5 per cent.		10 per cent.		20 per cent.		100 per cent.
	Selected	Rejected	Selected	Rejected	Selected	Rejected	Selected
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Selector A	40	84	50	86	45	89	18
,, B	10	92	25	93	20	94	9
,, C	50	87	50	89	35	90	15
A+B+C	10	98	15	99	10	100	2
A or B or C	60	73	70	76	60	79	29

$$\text{Selected} = \left(\frac{\text{Selection by eye}}{\text{Selection by weight}} \times 100\right) \qquad \text{Rejected} = \left(\frac{\text{Rejected by eye}}{\text{Rejected by weight}} \times 100\right)$$

(3) *Effect of selection by eye in F_2 on yield in F_3*

In four of the five families, the mean yield per plant of the progenies of plants selected in F_2 was above the mean yield per plant of the unselected family. The varying intensity of selection between F_2 plots

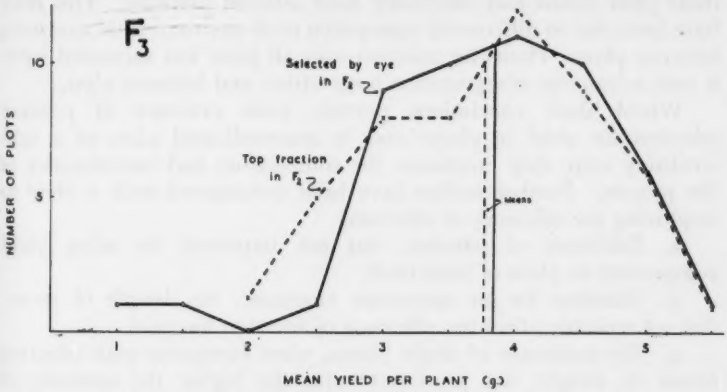

FIG. 4.—Distributions of the F_3 plots selected by eye in F_2 and an equal number selected by measurement in F_2.

is effective in raising the mean yield of the selected progenies above the over-all mean (*cf.* p. 226 (*c*)). The distribution of the progenies of F_2 plants selected by eye and by measurement are shown in fig. 4.

(4) *Effect of selecting in F_3 the progenies of selected F_2 plants*

In the previous section all plots were examined, including those whose parent plants had been rejected in F_2, and a number of such "rejected" plots have been selected in F_3. We now must determine whether selection between plots grown from selected F_2 plants resulted in real progress. Such selection was handicapped by the fact that all plots were grown in consecutive order, "F_2 selected" being mixed at random among "F_2 rejected," thus making comparisons within "F_2 selected" more difficult. Nevertheless, the mean yield of reselected F_3 plots is higher than that of F_3 plots grown from all selected F_2 plants.

	Mean yield per plant of F_3 plots	Number of plots
Unselected F_3 population	3·50	200
All plots selected in F_2	4·11	57
All plants selected in F_2	3·84	49
Plants selected in F_2, plots rejected in F_3 .	3·47	28
Plants selected in F_2, plots selected in F_3 .	4·29	21

CONCLUSION AND SUMMARY

1. In an F_2 from a compound cross, plotwise selection was successful, but plantwise selection within plots was successful only in those plots which had previously been selected plotwise. This may have been due to differential segregation or to environmental variation between plots. Plantwise selection over all plots was successful since it took advantage of segregation both within and between plots.

Whilst these conclusions provide some evidence of positive selection for yield in plants and in non-replicated plots of a self-fertilising crop, they emphasise the complexities and uncertainties of the process. Further studies have been commenced with a view to improving the efficiency of selection.

2. Efficiency of selection was not improved by using yield components in place of yield itself.

3. Selection for an agronomic character, viz. length of straw, did not seriously affect the efficiency of selection for yield.

4. Eye-judgment of single plants, when compared with selection based on weight, was more successful the higher the intensity of selection (table 2). Eye selection of plots was not as successful as that of plants (table 4).

Such comparisons are apt to reveal personal bias. The results show that eye selection by more than one observer raises considerably the efficiency of selection.

5. Selection by eye-judgment was as successful as selection by weight in raising the mean yields of the progenies.

6. The progenies of selected F_2 plants which were selected in F_3, gave higher mean yields than the progenies selected in F_2 but rejected in F_3.

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CONTAMINATION OF SEED CROPS

II. WIND POLLINATION

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Received 17.iv.47

CROSS-POLLINATION of seed crops by insects, dealt with earlier in this series (Bateman, 1947), may have seemed to seed growers a difficult problem. Cross-pollination by wind has generally been considered even more difficult to eliminate. Incidentally air-pollination is a more accurate term than wind-pollination as all air movements are effective in distributing air-borne pollen. One can observe insects at work pollinating a crop and their generally regular behaviour immediately impresses one. On the other hand, air currents are invisible, more erratic, and could conceivably carry pollen much farther than insects. Hive bees, by being deprived of all sources of nectar over a large area, can be made to fly up to seven miles from the hive (Eckert, 1933). According to the same author, however, they rarely fly more than two miles from the hive when an ample food supply is near at hand, and any one bee confines herself to a much smaller area than that bounded by a circle of two miles radius. Wind, on the other hand, continues indefinitely, and it is conceivable that individual pollen grains could be carried round the world, though it is most doubtful whether they would be viable after such an ordeal.

In fact, atmospheric pollen has been found at sea-level at all points across the Atlantic ocean between Sweden and New York. Erdtman (1943) analysed the air in mid-ocean during early summer by means of a vacuum cleaner, and found the concentration of pollen grains to be 0·7 grain per 100 cubic metres. At this point the commonest genera were Betula and Pirus. The comparable figure for the Swedish mainland was 18,000 grains per 100 cubic metres.

In view of these differences between insects and wind in the possible maximum distances over which they could carry contaminant pollen, it was thought desirable to carry out experiments on the effects of distance on contamination in two wind pollinated crops, beet and maize.

Beet, though generally regarded as wind pollinated, is probably to some extent pollinated by flies. In open flowers a disc, moist with nectar, is plainly visible, between stamens and style. The anthers, though hinged, are not versatile and flies of various kinds, including hover flies, can be seen visiting these flowers, though they do so

erratically and spend much of their time walking over other parts of the inflorescence searching for nectar. In 1944 an experiment was carried out, designed to assess the relative importance of wind and insects in pollinating spinach beet. Cages of various kinds constructed to exclude wind and flies, wind alone or flies alone, were placed over individual plants; but all caged plants died, even those in which the cage was open at the top.

EXPERIMENT I—BEET

Spinach Beet and red beet of the variety Crimson Ball were used, for with the former as seed parent, hybrids can be recognised at a very early stage among the progeny. Even the radicle emerging from the germinating seed is suffused with the anthocyanin introduced by Crimson Ball pollen.

The first attempt was a failure. The two varieties were sown at the same time in one year and planted out in the following spring in an arrangement similar to that described in the previous paper for turnips (Bateman, 1947). There was a square of Crimson Ball with arms to east and west three rows wide of Spinach Beet. The two varieties scarcely overlapped in flowering time, Spinach Beet bolting much earlier than Crimson Ball. Consequently, of the thousands of test progeny grown, only one contaminant seedling was observed. This served to emphasise the importance of isolation in time as a means of preventing contamination.

The following season the Crimson Ball was sown a month earlier than the Spinach Beet. In this way a greater degree of overlapping was obtained than the year before. The Crimson Ball was, however, much poorer in growth than the Spinach Beet. The central contaminating plot consisted of 169 plants of Crimson Ball. There were two arms of Spinach Beet, running north and south, each consisting of three rows. All plants were 9 inches apart each way. The north and south arms had 87 plants and 102 plants per row respectively. The three rows were designated *a*, *b* and *c*, *a* being the eastern and *c* the western row. The first five plants of each row were harvested and after that every fifth plant. All seed from each plant was harvested and a representative sample sown in 12-foot drills so that up to 400 seedlings were obtained from each tested plant. Some plants produced a very poor seed crop, so for each distance the results from the three rows were pooled and the contamination calculated from the pooled date (see table 1).

It will be seen at once that the maximum contamination is very small, about 10 per cent. This is probably due to the fact that in spite of their longer growing period the Crimson Ball plants produced much smaller inflorescences than the Spinach Beet. Their pollen would therefore be swamped by the Spinach Beet. There is no apparent difference between contamination in the two arms though

one might expect some in a wind-pollinated crop. The similarity between the general distribution of contamination in this and in the

TABLE 1

Distance (unit is 9 inches)	S series			N series		
	Hybrid	Total	F	Hybrid	Total	F
1	38	373	·1019	54	532	·1015
2	26	334	·0778	15	244	·0615
3	1	3	·3333	12	293	·0410
4	7	301	·0233	10	443	·0226
5	6	245	·0245	8	263	·0304
10	7	331	·0211	3	304	·0099
15	1	268	·0037	5	595	·0084
20	3	205	·0146	5	586	·0085
25	2	185	·0108	0	6	·0000
30	0	392	·0000	0	7	·0000
35	0	29	·0000	2	496	·0040
40	0	136	·0000	1	470	·0021
45	0	28	·0000	2	419	·0048
50	0	10	·0000	0	409	·0000
55	0	142	·0000	0	212	·0000
60	0	475	·0000	0	8	·0000
65	0	197	·0000	1	455	·0022
70	1	236	·0042	0	79	·0000
75	0	108	·0000	0	255	·0000
80	0	434	·0000	0	531	·0000
85	1	791	·0013	0	671	·0000
90	1	419	·0024	...	...	...
95	0	376	·0000	...	...	...
100	0	271	·0000	...	...	...

Contamination figures in Experiment I over distances up to 75 feet. The actual sizes of the test progenies are given to show which results are the most reliable. F is used as the symbol for the proportion of hybrid seed.

previously discussed radish and turnip experiments (*loc. cit.*) is very striking in view of the differences in pollination mechanisms. There is the same rapid fall over short distances but with a residual amount persistent up to relatively large distances.

EXPERIMENT II—MAIZE

Though the role of insects in beet pollination is uncertain, there is no doubt about the identity of the pollinating agent in maize. Bees often visit the tassels to collect pollen but they do not visit the silks.

A plot was laid out parallel to Experiment I. The contaminant plot consisted of 144 plants of Early Yellow Flint, 11 feet square with 1 foot spacing each way. The stringers consisted of a single row of Canada Gold with 2 feet spacing. Using these varieties contamination is measured as the proportion of flinty seeds on the Canada Gold ears. The *N* stringer extended for 84 feet, the *S* stringer for 78 feet. Though the two varieties overlapped to a great extent in flowering period, the flint corn was slightly earlier than the sweet corn. Canada Gold, being an open pollinated variety, showed

considerable individual variation in flowering time and in the interval between tasselling and silking. This meant that, cutting right across the variation in contamination due to isolation distance, there was variation due to earliness of silking and the degree of protandry. The latter is important in a sparsely planted stringer such as was used, because of its determination of the amount of non-contaminant pollen available at silking. There might also be expected variation between the cobs on one plant arising from their sequence of silking. On the other hand, the non-uniformity in time of tasselling of the Early Yellow Flint variety only serves to provide a steadier and longer supply of contaminant pollen, thereby reducing the error variation of contamination.

To provide a check on any variation not due to isolation distance, periodic surveys were made to obtain approximate dates of pollen shedding (not tasselling) and silking of all Canada Gold plants. Also, when harvesting, made a note of the position of the ear on the plant. This provides a record of the order of flowering since the highest ear on the main axis is the one which has silked first the tillers being always later. Where tillers produced terminal female or hermaphrodite ears instead of tassels this was also recorded.

TABLE 2

Distance from contaminant in feet	Plant no.	North stringer	South stringer	Dist.	Plant no.	North	South
2	1	*·7014	...	44	22	·0000	·0000
4	2	·0667	*·5359	46	23	·0000	*·0091
6	3	*·5724	...	48	24	*·0275	·0000
8	4	*·3222	*·4552	50	25	·0000	*·0000
10	5	·1170	·0127	52	26	*·0106	*·0101
12	6	·0324	...	54	27	·0240	·0000
14	7	·0075	*·0272	56	28	·0000	·0000
16	8	·0151	*·1042	58	29	*·0096	·0000
18	9	·0000	·0118	60	30	*·0120	*·0145
20	10	*·4190	·0081	62	31	*·0270	·0000
22	11	·0038	·0000	64	32	...	*·0032
24	12	...	...	66	33	...	·0000
26	13	*·1231	*·0207	68	34	...	0000
28	14	*·0354	*·0616	70	35	*·0163	*·0000
30	15	*·0610	*·0317	72	36	*·0031	*·0300
32	16	·0062	·0000	74	37	*·0036	*·0000
34	17	...	*·0132	76	38	*·0159	·0000
36	18	·0000	*·0063	78	39	*·0000	*·0000
38	19	*·0659	·0000	80	40	...	...
40	20	·0000	·0000	82	41	·0060	...
42	21	*·0578	·0000	84	42	·0000	...

Figures for contamination in Experiment II. Results marked with a * are those obtained from plants which silked before 11th August. Gaps are due either to the death of the plant or to obstructions (64-68 in the N series).

Table 2 shows the contamination per plant as a result of pooling all ears sufficiently mature to be scorable. It is immediately obvious

that there is a large error variation. Table 3 shows the times of pollen shedding and silking of all plants in both stringers. Comparison

TABLE 3

First observation of pollen-shedding

	31/7	5/8	9/8	11/8	14/8	16/8	22/8
North stringer	10 15 18 21 24 26 27 29 31 35 36 38	1 4 14 19 30	3	6 13 37	5 8 16	20 22 23 41	2 7 11 25 28 39
South stringer	2 3 4 7 8 13 14 17 36	15 23 26 30 32 38	18 20 29 33 35 37 39	10 15 27	9 11 16 21 22 24	5 19 28 31	6 12 34

First observation of silks

	31/7	5/8	9/8	11/8	14/8	16/8	22/8
North stringer	38	1 15 27 35 36	4 10 19 21 24 29 31	3 13 14 26 30 37 39	5 6 18 20 22	8 16 41 42	2 7 11 23 25 28
South stringer	36	14 26 30	2 4 7 8 17 23 32 37	13 15 18 25 35 39	9 10 19 20 21 27 29 33 38	11 24 31	3 5 6 16 22 38

Experiment II. The numbers of plants are recorded under the dates when they were first seen to be shedding pollen and when they were first seen to be silking.

of the two tables shows 11th August to be a critical date. Plants silking after that date show very little contamination, but plants silking before that date show appreciable contamination. If separate curves are drawn for the two classes of plants the effect of distance is seen to be much more regular and the error variance has largely disappeared. This is seen in fig. 1. Here it can be seen that contamination is generally higher in the *N* stringer than in the *S* stringer. The prevailing wind during the flowering period was south-west, so the cause of the difference is obvious.

There still appear to be a few anomalous plants. Examination of their pollen shedding and silking records, however, throws considerable light on their behaviour. For example, Plant *N* 10 gives too high a contamination (0·4190). It had already begun to shed its pollen on 31st July, but the first silks did not appear until 9th August. Thus self-pollination is almost ruled out. The nearest plants shedding pollen on 9th August were *N* 14 to leeward and *N* 4 to windward. Another plant with anomalously high contamination is *S* 36. This had its silks out on 31st July when there was very little Canada Gold pollen about, though Early Yellow Flint was shedding much pollen.

As in all previous experiments, over short distances there is a rapid fall in contamination with increasing distance, though the fall then continues gradually as the isolation distance becomes larger. Though great weight cannot be attached to it at this stage, it appears

that the contamination continues to decrease with increasing distance at all distances in the experiment. As maize is the only completely wind-pollinated crop dealt with so far, this is possibly of some importance. It might be traceable to a basic difference in the modes of action of insects and wind in pollination.

Where the requisite ears were available, an analysis of variance was carried out to test the effect within the plant of time of silking on contamination. The first ears on the main axis had greater contamination than the later ears on the main axis, the first ears on the tillers and the terminal cobs on the tillers. Though none of these

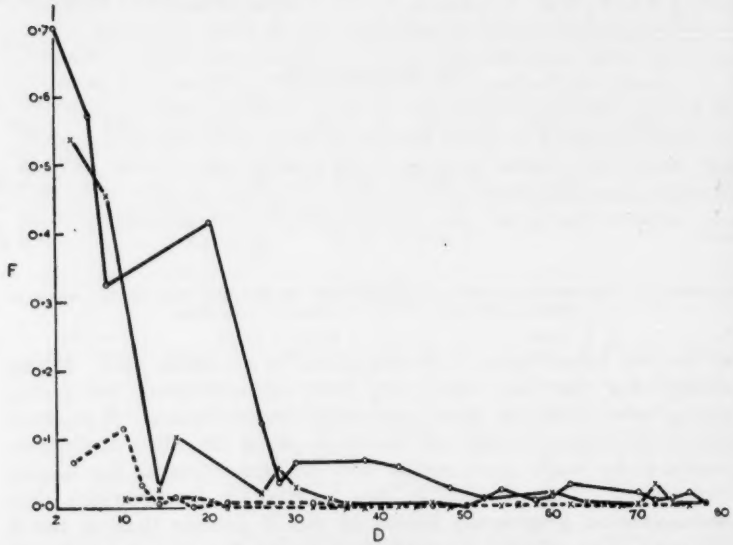

FIG. 1.—Experiment II. Graph showing relation between contamination F, and isolation distance in feet, D.

o———o N stringer silking before 11th August
×———× S ,, ,, ,, ,, ,,
o - - - o N stringer silking after 11th August
× - - - × S ,, ,, ,, ,, ,,

differences were significant, they were all in the direction to be expected from the effect of time on the contamination of individual plants.

In spite of the thin spacing (a single row of plants 2 feet apart) there appeared to be an adequate pollen supply, as most of the ears showed a seed set of 90 per cent. or more. This was so even at the ends away from the contaminant plot. There were a few exceptions which proved the rule. Ten plants of Canada Gold had a seed set of less than 70 per cent. Of these five produced their silks before

5th August and three on or after 22nd August, that is, before or after the main production of Canada Gold pollen. The two remaining plants were badly attacked by smut.

The weather conditions were generally dull and damp throughout the flowering period, which may have adversely affected the dispersal of the pollen. This would raise the percentage set in a sparsely planted plot, and would also reduce the extent of contamination.

EXPERIMENT III—POLLEN DISPERSAL IN MAIZE

One may perform an experiment on contamination in a wind-pollinated crop like maize, in such a way that variability of the contaminated variety in tasselling, silking, etc., is completely eliminated as a source of error. This was done by dispensing with the contaminated variety. In other words the plants exposed to contamination were replaced by slides coated with gelatine all exposed at the same height above ground, at the same time and for the same period, the only variables being the distance and direction from the source of pollen.

The density of the deposit of maize grains on the slide was measured by adding to the slide a drop of glycerine containing basic fuchsin, placing over it a ¾-inch square coverslip, and counting the number of maize grains under the coverslip. Though pollen of other species was deposited on the gelatine, maize grains were readily distinguishable if only by their large size.

A series of exposures was made for a number of days under varying meteorological conditions to assess the effects of rain, wind velocity and direction, etc. In this experiment, at each site two slides were exposed back to back in an erect position, as shown in the inset to fig. 2, 3 feet above ground level and protected from the rain by a small tin lid. With the exception of site O, one slide faced towards the centre of the maize block and one slide faced away from it. This arrangement would throw some light on the effect of atmospheric turbulence on pollen dispersal, *i.e.* the deviation of the paths of flight of pollen grains from straight lines.

Fig. 2 shows the arrangement of the exposed slides, the pollen source, and the position of wind breaks. The five exposures were made at the times and under the conditions shown in table 5.

The results for each distance in each stringer for each of the five exposures are shown in table 4. For the sake of clarity the sum of the two back-to-back slides is given for each site. In the Grand Total the figures for site o have been multiplied by 3. These results are shown graphically in fig. 3. In order, however, to show the variation between the two slides, the grand totals for all stringers are partitioned into (*a*) the number of grains on the side facing the pollen source, and (*b*) the number of grains on the other slide of the pair. For site o at the centre of the source (*a*) and (*b*) have been chosen

arbitrarily, (*a*) being westward facing. At sites 1, which are just within the borders of the pollen source, (*a*) faces towards the majority of the plants and (*b*) towards a few of them. Therefore, though on the average (*a*) should have more pollen than (*b*), the relative amounts

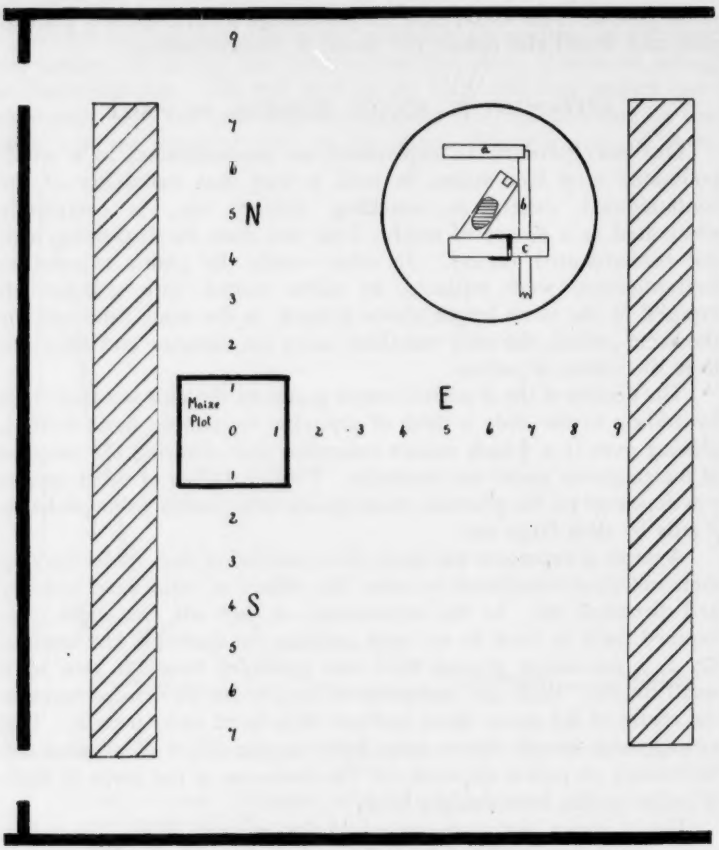

FIG. 2.—Experiment III. Plan of field showing arrangement of sites for exposures relative to maize plot. The thick black lines round the field represent a 10-foot wall. The hatched areas represent bush fruit trees. *Inset:* Diagram showing method of exposing slides; (*a*) protective tin lid; (*b*) 3-foot cane inserted in ground; (*c*) clothes-peg gripping cane and back-to-back slides on which the gelatined area is shown shaded.

in each case will vary with the wind direction. For this reason the difference between (*a*) and (*b*) is less marked at sites 0 and 1 than at the remainder, which clearly show that maize pollen tends to drift in straight lines and thus is only rarely deposited on the slide facing away from the source.

TABLE 4

Experiment III. The number of pollen grains per site per exposure.

Exposure		1	2	3	4	5	Site Totals
	0	1104	626	296	380	89	2495
Stringer E. . .	1	327	1036	410	199	161	2133
	2	18	210	71	7	21	327
	3	1	55	21	2	7	86
	4	1	32	17	0	6	56
	5	1	35	6	0	4	46
	6	0	14	4	0	4	22
	7	0	12	1	0	1	14
	8	0	6	0	0	0	6
	9	0	1	0	0	0	1
Total per exposure .	2-9	21	365	120	9	43	
Stringer N. . .	1	319	313	234	819*	116	1801
	2	39	16	3	9	11	78
	3	2	0	2	0	5	9
	4	2	1	0	0	3	6
	5	2	0	0	1	0	3
	6	0	1	0	0	0	1
	7	0	0	0	0	0	0
		—	—	—	—	—	—
	9	0	0	0	0	0	0
Total per exposure .	2-9	45	18	5	10	19	
Stringer S. . .	1	1335	971	343	592	155	3396
	2	125	271	91	174	7	668
	3	43	95	31	76	1	246
	4	17	37	8	29	0	91
	5	10	16	7	39	0	72
	6	2	12	2	17	0	33
	7	3	3	1	9	0	16
Total per exposure .	2-7	200	434	140	344	8	
Deduced wind direction .		N.E.	N.W.	N.W.	N.E.	S.W.	
Total grains counted per exposure		3351	3763	1548	2353	591	

Site	Grand total	Partitioned	
		(*a*)	(*b*)
0	7485	3156	4329
1	7330	5230	2100
2	1073	1057	16
3	341	338	3
4	153	151	2
5	121	119	2
6	56	54	2
7	30	30	0
(8)	6	6	0
(9)	1	1	0

The grand total is the sum of all stringers and all exposures for each distance and is partitioned into (*a*) slide facing towards maize block and (*b*) slide facing away from maize block. For comparison with the others the 0 total has been multiplied by 3 in the Grand Total figures.

* Slide N1, exposure 4, showed clumped grains as might arise if birds had scattered pollen on to it.

TABLE 5

No.	Time and duration	Wind force	Average direction	Maximum temperature °F.	Rain
1	11 a.m. 25/7—for 24 hrs.	Slight wind	N.E.	73	trace
2	11 a.m. 28/7—for 48 hrs.	Steady breeze	N.W.	72	trace
3	11 a.m. 31/7—for 24 hrs.	Slight breeze	N.W.	71	none
4	12.30 2/8—for 24 hrs.	Slight wind	N.E.	78	none
5	12.30 7/8—for 24 hrs.	Strong wind	S.W.	56	·07 in.

From the totals for sites from 2 upwards in each arm we can deduce the mean wind direction during each exposure.

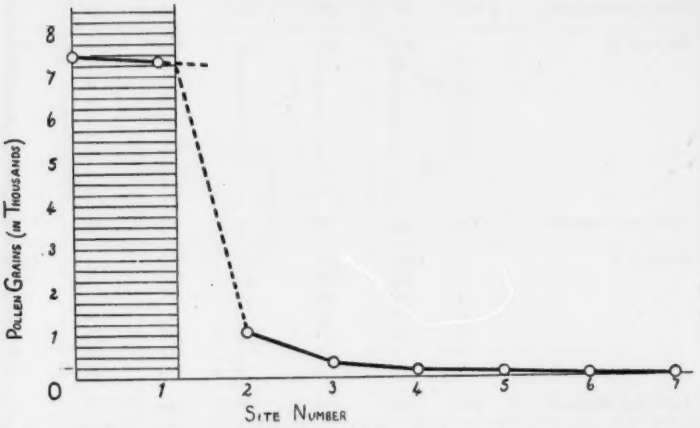

FIG. 3.—Experiment III. Total numbers of pollen grains for all exposures and all three stringers at each distance from the centre of the maize plot. For comparison the number of grains at site 0 has been multiplied by 3. The shaded area represents the maize plot.

As to the general effect of distance on pollen distribution or contamination, these results show, with much less error variation than Experiment II, that maize pollen is not carried very far. With regular increases in isolation distance the dangers of contamination will diminish rapidly at first, but more and more slowly as the isolation distance becomes greater.

CONCLUSIONS

Neither beet nor maize are typical wind-pollinated species. Beet has members of the Diptera as supplementary pollinating agents. Maize has excessively large grains which are consequently less buoyant than grains of other anemophilous plants. They will not therefore be distributed so widely by a given wind.

Even in the grasses, however, with very buoyant pollen, one would expect the curve relating contamination to distance to be of the same basic shape as in maize, the difference residing in the slope. This is shown in a large series of experiments carried out by Jensen and Bøgh (1941). They observed the numbers of pollen grains deposited on vaseline-smeared slides exposed *down-wind* from large plots of the following crops : rye grass, timothy grass, cocksfoot, rye and sugar beet. In all cases the distances involved were very much greater (0-1200 metres) than in the above experiments.

In the cases of rye grass and cocksfoot there is an appearance of a minimal value beyond 500-600 metres of about one-twentieth the concentration at the source. This might represent the general level in the atmosphere due to other sources, which would be increased by misclassification of pollens which might well arise in the grasses. If, however, we allow for the difference in scale of the distances for grasses and sugar beet and for maize the shape of the frequency curve is strikingly similar in Jensen and Bøgh's diagram and in the maize results published herein.

In Experiment III of the previous paper in this series (Bateman, 1947) it was shown that the smaller the mass of the contaminated variety the more gradual was the slope of the curve. Increased buoyancy of pollen would have an effect similar to that of decreased mass. It is reasonable to suppose, therefore, that the effect of buoyancy in grass pollen could be counteracted by increasing the mass of the variety.

In spite of the differences between the pollination behaviour of maize and beet and between both of these and the entomophilous species dealt with earlier (*loc. cit.*), they all show substantially similar relationships between contamination and isolation distance. In every case a great reduction in contamination is produced by the first ten to twenty feet, and though contamination continues to decrease with increasing isolation distance beyond this point, the rate of decrease becomes steadily smaller. We can conclude, therefore, that though significant differences between wind-pollinated and insect-pollinated plants may be disclosed later, the evidence so far does not justify the use of isolation distances of different orders of magnitude in these two kinds of crop.

SUMMARY

Beet and maize were used for a study of the influence of distance in reducing cross-pollination, *i.e.* in effecting isolation in wind-pollinated crops, under conditions conducive to cross-pollination.

In beet, Carter's strain of Spinach Beet was exposed to contamination from red beet of the variety Crimson Ball. The maximum contamination (at distance zero) was low (10 per cent.) because of the relatively small inflorescences of the red beet. With increasing

distance, contamination rapidly declined. At 30 feet it was 1 per cent. of the maximum.

In maize, Canada Gold (sweet corn) was contaminated by Early Yellow Flint, contamination being discernible on the ripe ear. The results were similar but there was a distinct variation in contamination with direction from the contaminant, variation attributable to the wind direction being mainly south-west. One per cent. contamination was observed at 50 feet in the northern direction and 40 feet in the southern direction.

In both crops the rate of decrease in contamination steadily decreased with increasing isolation distance.

A high error variance between plants of Canada Gold was found to be due to variation in their silking time (female flowering). Late-silking plants missed the main flowering period of the contaminant variety, and therefore had less contamination than earlier plants at similar distances.

In the following year the use of glycerined slides to trap pollen grains around a maize plot produced evidence completely corroborating the previous contamination results. Here the time factor was eliminated from error variation. A distance of 60 feet was sufficient to reduce atmospheric pollen to 1 per cent. of the concentration obtaining within the plot.

The results of other workers on pollen dispersal in herbage grasses and beet, after due allowance has been made for a greater buoyancy of pollen in these crops, agree with the present maize results.

One may conclude that the requirements in respect of isolation distance of wind-pollinated and insect-pollinated crops are similar. The greater buoyancy of the pollen of some wind-pollinated crops could be allowed for by increasing the size of plots liable to contamination.

Acknowledgment.—This work has been carried out under the auspices of the Agricultural Research Council. The author also wishes to express his gratitude to Dr K. Mather for his advice.

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SALTANT PRODUCTION IN THE FUNGUS *CHÆTOMIUM GLOBOSUM* BY ULTRA-VIOLET LIGHT, AND ITS RELATION TO ABSORPTION PROCESSES

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Received 27.xii.46

INTRODUCTION

It has been shown that a certain well-marked saltant of the fungus *Chætomium globosum*, which has been called K, is produced selectively in large numbers by some ultra-violet frequencies and not at all by other frequencies, although in all experiments other saltants are freely produced (McAulay, Plomley and Ford, 1945 ; and Ford, 1946*b*).

The present paper gives an account of the further investigation of the production of this K saltant at wave lengths near the threshold, and of the way in which the frequency of production of K and other saltants varies with wave length.

The relationship between the saltant production curve and absorption curves of nucleic acid and proteins is discussed. It is shown that it is difficult to reconcile the results with the conclusions of Knapp and Schreiber (1939) and others, who believe that mutations produced by ultra-violet radiation are the result of a primary absorption by nucleic acid. On the other hand, all the phenomena observed can be explained in terms of protein absorption if this is looked on as the result of more than one excitation in the protein molecule.

EXPERIMENTAL METHOD

The source of the ultra-violet and visible frequencies was a commercial mercury vapour discharge lamp (McAulay, Plomley and Ford, 1945), and the large aperture monochromator used for most of the experiments has been described by McAulay and Taylor (1939). A Hilger (D33) monochromator was used to improve the resolution in the neighbourhood of the threshold for production of the K saltant. The Hilger monochromator gives a purer spectrum than the other, but one of much lower intensity. Intensity was measured by means of a vacuum thermopile and a low resistance galvanometer. Considerable difficulty was encountered in obtaining absolute values with the large aperture monochromator on account of the rapid divergence of the beam, and no great accuracy is claimed for them. Relative values are considerably better.

For wave lengths greater than 3132 A.U. only the large aperture monochromator was used, as the time involved in irradiation was

about twelve days at 4047 A.U. even with this monochromator. For wave lengths of 2967 A.U. and shorter, experiments have been made with this instrument as well as with the Hilger. Glass filters (see also McAulay, Plomley and Ford, 1945; and Ford, 1946 (*b*)) were used to prevent contamination of long wave lengths by short wave lengths. When using the Hilger monochromator the spectral line was isolated by a slit placed immediately in front of the experimental spores, and the controls were placed in the line of the experimental spores, just beyond the limit of the spectral line. In this way they served as a check on any radiation of other wave lengths that might be irregularly reflected from the spectrometer telescope. In experiments on the 4047 line with the large monochromator the controls were situated in the yellow line of the mercury spectrum so that they were subjected to a greater dose of longer wave length radiation than the experimental material.

The filters varied from a fine corex glass coverslip at 2804 A.U. to five microscope slides at 3656 A.U., and three thicknesses of Crookes glass at 4047 A.U., the visible violet line.

The method of spreading and irradiating the spores and subsequent single spore and dilution plate techniques have been described by McAulay, Plomley and Ford (1945). The fungus used was *Chætomium globosum* (Ascomycetes, Sphæriales).

Approximately 2000 spores for both experimental and control series were spread on coverslips for each experiment.

Colonies were of single spore origin and each colony was grown in a separate petrie dish. When the colonies were three or four weeks old an analysis was made of the saltants produced. McAulay, Plomley and Ford (1945) describes the classification of saltants, and Ford (1946*a*) describes the selectively produced saltant " K."

To determine the percentage of the radiation absorbed by the spores at wave lengths 2654 A.U. and 2804 A.U. a photographic method was employed. A layer of spores, one spore thick, was spread behind a pin-hole 0·2 mm. in diameter on a quartz coverslip. With care, an area of this size could be selected with a uniform layer of spores. Positive film was exposed behind this pin hole in close contact with the coverslip. The density of the film behind the spores was matched against film exposed for a suitable time directly to radiation of the same wave length.

RESULTS OF IRRADIATION

Saltants have been produced by six wave lengths of ultra-violet light, 2654 A.U., 2804 A.U., 2967 A.U., 3132 A.U., 3342 A.U. and 3656 A.U., and by the visible violet line 4047 A.U. Some of the experiments have been described in previous papers, and a full description of the saltants produced has been given (McAulay, Plomley and Ford, 1945; and Ford, 1946). The table collects data in which

one particular parent material was used. This was itself a saltant of *Chætomium globosum* named Fld. It has characteristics which make it particularly suitable for experimental purposes. Results obtained with other material were consistent with those set out, but this table summarises the most complete and uniform set of experiments.

TABLE

Saltants produced by monochromatic ultra-violet frequencies 2654 A.U., 2804 A.U., 2967 A.U., 3132 A.U., 3342 A.U. and 3656 A.U., and by the visible violet wave length 4047 A.U. in colonies grown from spores of the fungus Chætomium globosum Fld, *and dose required to produce them.* (*Experiment roman, control italic*)

Wave lengths	2654 A.U.	2804 A.U.	2967 A.U.	3132 A.U.	3342 A.U.	3656 A.U.	4047 A.U.	Total all wave lengths
Number of experiments	10	8	5	9	9	6	7	54
Number of colonies from single spores	986 *450*	614 *284*	644 *239*	947 *433*	1272 *587*	616 *303*	775 *302*	5854 2598
Number of K saltants .	254 *0*	170 *0*	89 *0*	23 *0*	18 *0*	1 *0*	1 *1*	566 *1*
Number of other saltants	81 *3*	43 *4*	36 *0*	44 *3*	47 *0*	24 *1*	26 *2*	301 *13*
Percentage of K saltants in total number of colonies grown	25·75 *0*	27·7 *0*	13·8 *0*	2·4 *0*	1·3 *0*	0·16 *0*	0·1 *0·3*	
Percentage of other saltants in total number of colonies grown	8·2 *0·7*	7·0 *1·4*	5·6 *0*	4·6 *0·7*	3·7 *0*	3·9 *0·3*	3·4 *0·6*	5·2 *0·5*
Ratio of K saltants to others	3·1 *0*	3·96 *0*	2·5 *0*	0·52 *0*	0·35 *0*	0·042 *0*	·038 *·05*	
Dose in joules/cm.^{2} .	0·52	0·24	0·9	30	150	900	1800	

The table shows the number of experiments performed, the total number of colonies, the number of saltants produced, and the dose for each wave length. A comparison is made with control colonies. All colonies were of single spore origin.

The most striking feature of the ultra-violet experiments is the large number of K saltants (fig. 1) produced by short wave lengths, 2654 A.U., 2804 A.U. and 2967 A.U. More than three times as many K's are produced at these wave lengths as all other saltants put together. Few are produced by 3132 A.U. and 3342 A.U., and practically none by 3656 A.U. and by the visible line 4047 A.U., although under the conditions of high dosage employed the production of other saltants was of the same order as at short wave lengths. It is suggested that the total of three K saltants found in experiments at 3656 A.U., 4047 A.U., and in control are due to stray ionisation. The K saltation is an easily recognisable saltant and has been described in detail by Ford (1946*a*).

Saltants other than K have been classified previously (McAulay, Plomley and Ford, 1945) into three main groups, depending on differences from the normal (fig. 2) in growth rates, form, production

and distribution of perithecia, and mycelial characters. Two saltants are shown in fig. 3. The number of these saltants, other than K, produced by the six frequencies of ultra-violet and the one visible line has been tabulated in the fourth row in the table in this paper. It is possible that in this large group of saltants there are included some which are also produced selectively. The K saltation has been separated from others in the table on account of its selective production.

In the fifth row of the table the percentages of colonies grown

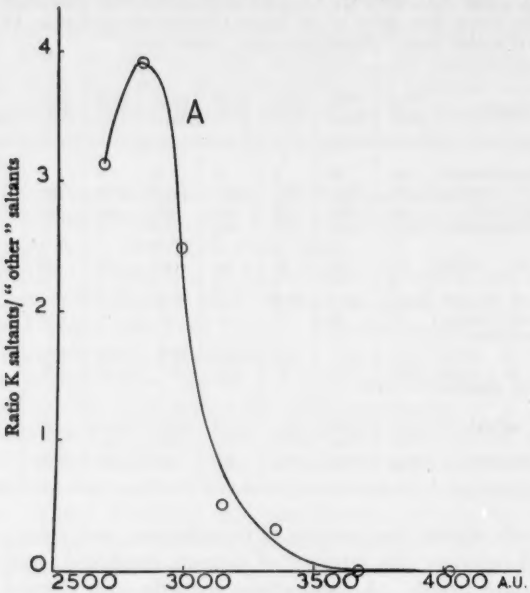

FIG. 4.—Curve A shows the ratio of percentage K saltants to the percentage " other " saltants (see the table), and curve B shows the optimum doses in joules per sq. cm., for saltant production. Both curves are plotted against wave length. Curve A is natural scale and curve B is plotted on a logarithmic scale. The doses read downwards to bring out the similarity between curves A and B.

from irradiated spores which became K saltants are shown for different wave lengths. In the sixth row the same percentages are given for all other saltants grouped together. In row seven the ratio of the K percentage to the " other " saltant percentage is tabulated and the result is plotted in curve A (fig. 4). These ratios and the graph show most clearly the contrast between the selective production of K and the production of other saltants.

The last row in the table gives the average doses required at the different wave lengths to produce the effects above described. These doses are plotted in curve B (fig. 4) on a logarithmic scale. The doses read downwards to bring out the similarity between curves A and B.

Two strains of *Chætomium globosum*, Kb and Lj (McAulay, Plomley and Ford, 1945), and another *Chætomium globosum* saltant, St (McAulay, 1938), have been tested for the production of saltants by ultra-violet frequencies. The resulting saltants have been similar to those found by irradiating *C. globosum* F*l*d spores, but as F*l*d was a proved pure strain and convenent for experiment the others were discontinued.

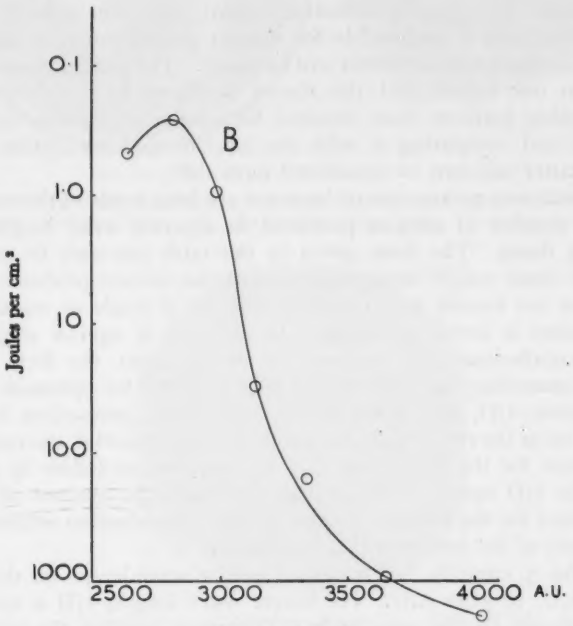

FIG. 4.—Curve B.

A few experiments were made with *Chætomium elatum*, another species of Chætomium, and the K saltation and " other " saltants were also produced by short ultra-violet irradiation of the spores.

GENERAL DISCUSSION OF RESULTS

The results of the present investigation are of interest in the light of the opinion stated by a number of authors (Knapp and Schreiber, 1939; MacKenzie and Muller, 1940; Stadler and Uber, 1942) that mutations produced by ultra-violet light are primarily caused by an absorption process in nucleic acid. Knapp and Schreiber, and Stadler and Uber both experimented with plant material, and the relations they found between mutant production and wave length fit very closely the absorption curve of nucleic acid. A similar fit was found by Emmons and Hollaender (1939) experimenting with a

fungus. Our results are entirely different, and it would seem that a different explanation should be given for the production of the fungal saltants that we have investigated.

Two quite separate features of our results are inconsistent with the nucleic acid absorption theory. The first is qualitative and indirect. It arises from the clear-cut selective effect that we have recorded, K saltations being produced by some wave lengths and not by others. Knapp and Schreiber (1939) point out that if nucleic acid absorption is responsible for mutant production it is not to be expected that a selective effect will be found. The second inconsistency between our results and the theory is shown by considering the relationship between doses required for saltant production and wave length, and comparing it with the nucleic acid absorption curve. This matter will now be considered more fully.

Satisfactory measurements have not yet been made of the variation in the number of saltants produced at separate wave lengths with varying doses. The doses given in the table can only be taken as roughly those which correspond to optimum saltant production, and it is not yet known with certainty whether a single or multiple hit mechanism is involved, though the evidence is against single hits. Some conclusions can, however, be drawn from the form of the curve connecting the reciprocal of dose required for optimum saltant production, 1/D, with wave length. If saltant production is to be looked on as the result of an absorption in some material, the extinction coefficient for the absorption may be expected to follow in general form the 1/D curve. This assumes that the same amount of energy is required for the primary process in saltant production whatever the frequency of the radiation that supplies it.

In fig. 5, curve A, 1/D is plotted against wave length for the range 2654 A.U. to 3132 A.U. For longer wave lengths 1/D is too small to be shown on the same scale. The curve relating the extinction coefficient for nucleic acid absorption taken from Caspersson (1936), curve B, is shown for comparison. It is evident that the two curves do not fit. It is only possible to reconcile our results with this absorption as a primary cause of the saltations if very special conditions of screening exist in the spore. There is evidence that this is not the case. The matter will be discussed further below.

Curve C (fig. 5) shows the absorption curve for a protein, serum albumin, also taken from Caspersson. It is evident that in the spectral region 2654 A.U. to 2967 A.U. saltant production would be very satisfactorily explained by protein absorption of the albumin type.

For wave lengths longer than 2967 A.U. the 1/D curve falls more rapidly than the protein absorption curve, and referring to fig. 4, curve A, it will be seen that the number of K saltants decreases to zero, but the "other" saltants continue to appear in roughly the same numbers even into the visible spectrum, provided sufficiently large doses are given.

FIGS. 1-3.—Colonies of single spore origin. Saltants produced by 2654 A.U., a short ultra-violet wave length.

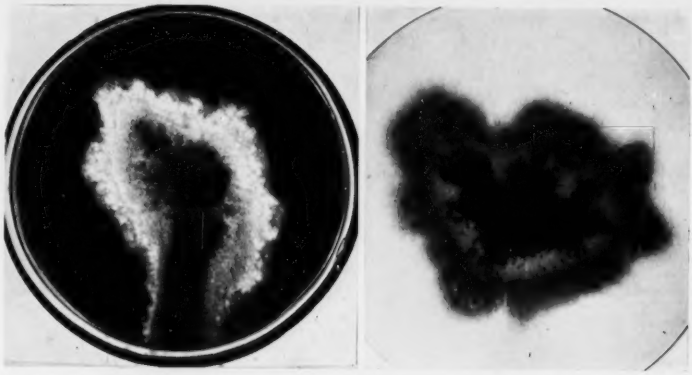

FIG. 1*a*. FIG. 1*b*.

FIG. 1.—Examples of "K saltation" colonies. (*a*) K saltant colony with one normal sector. Reflected light. ×¾. (*b*) Total K saltant colony. Transmitted light. ×1.

FIG. 2.—Normal colony of *Chætomium globosum* Fld. Reflected light. ×¾.

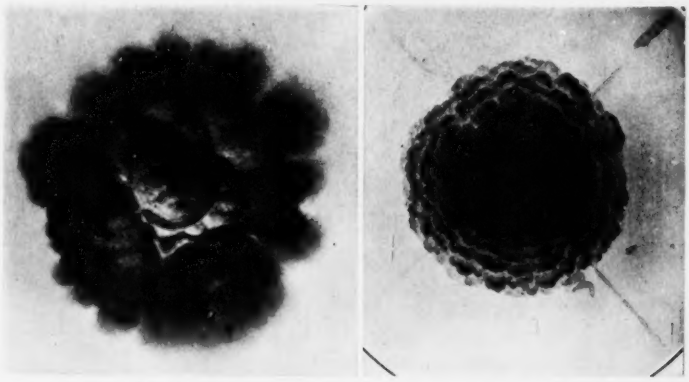

FIG. 3*a*. FIG. 3*b*.

FIG. 3.—Examples of "other" saltants. (*a*) Slow growing colony with no perithecia and dense mycelium. Reflected light. ×1¼. (*b*) Slow growing saltant with scalloped appearance and few perithecia. Transmitted light. ×1.

TENTATIVE PROTEIN ABSORPTION THEORY

The following theory accounts simply for the facts and will be tentatively adopted to give form to the experimental results. The particular change in some protein molecules that gives rise to the strong absorption band with maximum about 2800 A.U. is not excited beyond about 3300 A.U., and this change is the only one that can give rise to " K " saltants. It is also this change which is the most effective stimulus for " other " saltants, but it is not the only one that can produce them. The absorption of longer wave lengths

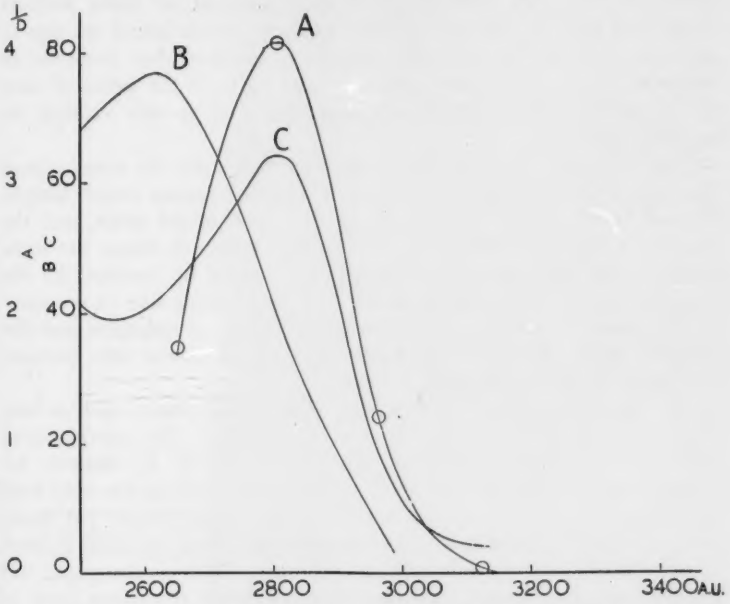

FIG. 5.—Curve A plots the reciprocal of optimum dose for saltant production against wave length. Curve B shows the extinction coefficient for nucleic acid absorption (after Caspersson, 1936), and curve C shows the extinction coefficient for a protein, serum albumin (also from Caspersson), plotted against wave length.

can produce saltants other than K, but a smaller proportion of the energy absorbed in this spectral region is effective for the purpose. For this reason the saltant dose curve (A, fig. 5), beyond about 3000 A.U. falls more rapidly than the protein absorption curve.

It is recognised that this theory is by no means the only possible one to account for the facts, but its simplicity makes it useful for summarising the results and suggesting further work.

THE EFFECT OF SCREENING IN CHANGING THE SHAPE OF A 1/D, λ CURVE FOR SALTANT PRODUCTION

It is possible, though unlikely, that the form of curve found by us connecting the reciprocal of dose for constant saltant production with wave length (curve A, fig. 5) could be due to a primary absorption in nucleic acid in spite of the difference in shape between it and the nucleic acid extinction coefficient curve. The difference in shape could be due to a screening of the sensitive spot by material with appropriate absorption characteristics, possibly even nucleic acid itself. This is a point of considerable interest as the reverse phenomenon might also be true, and frequency curves found by some authors resembling nucleic acid extinction coefficient curves might be due to absorption in protein with screening by a material that absorbed in the same way. The matter will be discussed from the point of view of our results and it will appear that this cause is very unlikely to account for them.

Curve A, fig. 6, shows the reciprocal of dosé for the same saltant production at different wave lengths plotted against wave leng'h. By dose in this curve is meant energy incident on the spore, and the curve is a direct statement of our results. Curve E shows the same curve where dose means the energy that would be received by the sensitive spot if a screen of thickness 10·3 μ absorbing like 11 per cent. nucleic acid were interposed between the source of radiation and the sensitive spot. It is derived from curve A by taking into account absorption in this postulated screen.

It will be seen that with this kind of screening results such as ours might be explained by nucleic acid absorption. The nucleic acid extinction coefficient curve is reproduced (curve B, dotted) for comparison. It is at once evident that screening by nucleic acid itself is unlikely to account for our results as the spore is only 7 μ thick, and according to Caspersson chromosomes in which the acid content is particularly high contain only 11 per cent. Our results might still be due to some other screening material with the same type of absorption curve as nucleic acid but with a higher extinction coefficient.

To test this point an attempt was made to obtain the relative absorption by a single layer of spores of radiation of 2654 A.U. and of 2804 A.U. The experiment proved difficult to carry out and the results obtained were subject to large errors. It was possible to establish, however, that the absorption in a single layer of spores was slightly greater for 2654 A.U. than for 2804 A.U., but that the ratio of the absorptions for the full spore thickness was very much less than 2 to 1. When it is remembered that the sensitive spot must lie within the spore and therefore that the ratio of the screening for 2654 A.U. and 2804 A.U. must be still smaller it will be seen that the general shape of our curve would not be altered if dose incident on the

sensitive spot replaced dose incident on the spore. About 99 per cent. of the total radiation incident on the spore is absorbed at these wave lengths.

It is still possible that measurement of absorption in the whole spore does not give a correct picture of the screening, that there might be a local very dense screen with nucleic-acid-like absorption character-

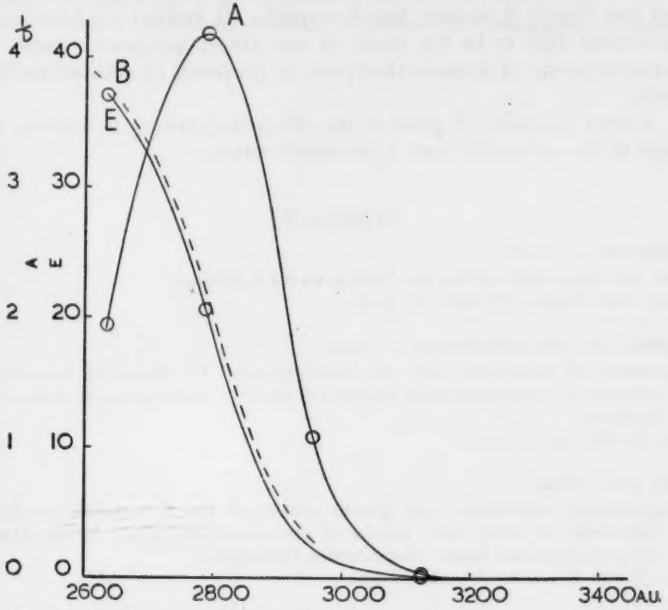

FIG. 6.—Curve A plots reciprocal of the dose incident on the spore (see also fig. 5, curve A) against wave length, and is a direct record of experiment. Curve E plots reciprocal of the dose reaching the sensitive spot on the assumption that the radiation shown in curve A is absorbed by a thickness of 10·3 μ 11 per cent. nucleic acid. Curve B (dotted) is the nucleic acid extinction coefficient curve (see fig. 5, curve B).

istics but of much greater opacity, which guards the sensitive spot. Such a theory seems to strain probabilities, and it is a great deal simpler to accept some such explanation as the tentative protein absorption theory proposed. This is particularly true when account is taken of the selective production of K saltants, which by itself casts serious doubt on a purely nucleic acid absorption theory.

SUMMARY

Experiments are described on the production of saltants by monochromatic ultra-violet irradiation of spores of the fungus *Chætomium globosum*. It is found that one saltant which is produced in large numbers by short wave ultra-violet irradiation is not produced by

long wave lengths. It is also found that the curve connecting the reciprocal of dose for optimum saltant production with wave length is inconsistent with the assumption that mutant production by ultra-violet radiation is due to absorption of the radiation in nucleic acid. This assumption has been found to agree with all results previously reported.

The fact that one saltant is produced selectively suggests strongly that any theory is suspect which requires all mutant production by ultra-violet light to be the result of one absorption process only. A tentative theory of protein absorption is proposed to account for our results.

A brief discussion is given of the effect of screening in altering the shape of the reciprocal dose wave length curve.

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CONTENTS

PAGE

FISHER, R. A. (Cambridge), and FORD, E. B. (Oxford). The spread of a gene in natural conditions in a colony of the moth *Panaxia dominula* L. 143

MATHER, K. (Merton). Species crosses in *Antirrhinum*.—I. Genetic isolation of the species *majus*, *glutinosum* and *orontium* 175

DARLINGTON, C. D. (Merton), and KOLLER, P. C. (London). The chemical breakage of chromosomes 187

BOYCE, S. W., COPP, L. G. L., and FRANKEL, O. H. (Christchurch). The effects of selection for yield in wheat 223

BATEMAN, A. J. (Merton). Contamination of seed crops.—II. Wind Pollination 235

MCAULAY, A. L., and FORD, JOAN M. (Hobart). Saltant production in the fungus *Chætomium globosum* by ultra-violet light, and its relation to absorption processes 247